obtained by acetylation of the reaction product to give 4-(acetoxyamino)quinoline (**6**), shown to be identical (HPLC, TLC) with an authentic sample.

Reaction in neutral or basic conditions. pH 7: the monoester solution was diluted with 10 mL of 1 N acetate buffer solution. A precipitate was immediately formed; it was filtered and analyzed by mass spectrometry: MS, *m/e* (relative intensity) 314 (7), 298 (10), 284 (15), 282 (100), 270 (11), 254 (7), 141 (11), 127 (16). pH 10: the precipitate formed was analyzed by mass spectrometry: MS, *m/e* (relative intensity) 332 (4), 316 (6), 314 (2), 300 (8), 298 (4), 284 (20), 282 (12), 271 (5), 255 (5), 160 (14), 158 (14), 149 (27), 145 (58), 144 (26). pH 13: the 4-(hydroxyamino)quinoline 1-oxide **2** intermediately formed was analyzed by HPLC; it decomposed in the reaction conditions to give a red solid, analyzed by mass spectrometry: MS, *m/e* (relative intensity) 330 (1), 316 (4), 316 (1), 300 (17), 298 (4), 284 (85), 282 (26), 255 (23), 144 (17), 129 (80). The mass spectrum was identical with the one obtained by dissolving synthetic 4-(hydroxyamino)quinoline 1-oxide (**2**) in the same solvent.

Registry No. **1**, 56-57-5; **3**, 18061-48-8; **4**, 84752-93-2; **6**, 32654-59-4; **7**, 117940-74-6; **8**, 13442-05-2; **9**, 58550-85-9; **10**, 2508-86-3; **11**, 19701-39-4; **13**, 117940-75-7; **14**, 19701-38-3; **15**, 117940-76-8; **18**, 53972-05-7.

Concerning Model Metabolites of the Carcinogen 4-Nitroquinoline 1-Oxide. Reactivity of 1-Acetoxy-4-(hydroxyimino)-1,4-dihydroquinoline

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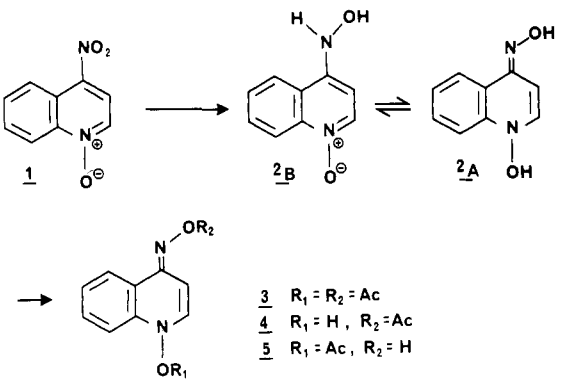
1-Acetoxy-4-(hydroxyimino)-1,4-dihydroquinoline is obtained quantitatively by hydrolysis of 1-acetoxy-4-(acetoxyimino)-1,4-dihydroquinoline under strongly acidic conditions. At pH <1 the monoacetate is protonated and on hydrolysis affords only 1-hydroxy-4-(hydroxyimino)-1,4-dihydroquinoline. Above pH 1 on hydrolysis the monoacetate affords a complex product mixture including 4-(hydroxyamino)quinoline and 4-nitrosoquinoline. Comparison of the above behavior of 1-acetoxy-4-(hydroxyamino)-1,4-dihydroquinoline with that of 1-hydroxy-4-(acetoxyimino)-1,4-dihydroquinoline at a neutral pH suggests that the former type of ester is less likely to be important in those steps initiating oncogenesis. In contrast the latter type of monoester or diesters corresponding to 1-acetoxy-4-(acetoxyimino)-1,4-dihydroquinoline by their ability to generate nitrenium ion intermediates and undergo attack by nucleophiles at the 3-position are likely intermediates in the process of oncogenesis. Further aspects of their likely biological role are discussed.

There has been great interest in the biology and the chemistry of 4-nitroquinoline *N*-oxide (**1**), which is one of the most potent and most studied of synthetic chemical carcinogens.[1] Reduction of the nitro group is a prerequisite for the initiation of oncogenesis by this compound.[2] Such a reduction may lead to the hydroxylamine **2**, capable of existing in the tautomeric forms $\mathbf{2}_A$ and $\mathbf{2}_B$.[3] It has been suggested that esters of the hydroxylamine **2** may be involved in the metabolic process leading to the observed oncogenesis.[4] As discussed in the accompanying paper,[5] these suggestions have stimulated our present work to understand the likely pathways open to diesters of the hydroxylamine **2**, such as the diacetate **3**, and to monoesters **4** and **5** (Scheme I). In the early part of our work,[6] we reported the behavior of the diacetate **3** and established that in different pH ranges the two monoacetates **4** and **5** were obtained as products. In the preceding paper[5] we report the hydrolysis of the monoester **4** and establish that under certain conditions solvolysis involves nitrenium ion intermediates. In this paper we now describe the quite different chemistry of the second monoacetate **5**. The reactivity of the monoester **4** is dominated by the activating effect of the *N*-oxide function. The absence of this effect in the monoester **5** leads to a contrast in the chemistry of the two series of monoesters. In the light of those results reported earlier[5,6] and in this paper, we are now able to comment further on the relationship between the chemistry of those derivatives of 4-nitroquinoline *N*-oxide, which have been postulated as metabolites, possibly involved in the initiation of oncogenesis, and the formation

Scheme I



† Université Joseph Fourier-Grenoble.
‡ The University of Southampton.

(1) Sugimura, T. In *Carcinogenesis, vol. 6: the Nitroquinolines*; Raven Press: New York, 1981.
(2) Okabayashi, T.; Yashimoto, A. *Chem. Pharm. Bull. (Tokyo)* **1962**, *10*, 1221.
(3) Kawazoe, Y.; Ogawa, O.; Huang, G. F. *Tetrahedron* **1980**, *36*, 2933.
(4) (a) Enomoto, M.; Sato, K.; Miller, E. C.; Miller, J. A. *Life Sci.* **1968**, *7*, 1025. (b) *Molecular Biology of Mutagens and Carcinogens*; Singer, B., Grunberger, D., Eds.; Plenum Press: New York, 1983; p 171.
(5) Demeunynck, M.; Lhomme, M. F.; Mellor, J. M.; Lhomme, J. *J. Org. Chem.*, preceding paper in this issue.
(6) Demeunynck, M.; Lhomme, M. F.; Lhomme, J. *J. Org. Chem.* **1983**, *48*, 1171.

0022-3263/89/1954-0405$01.50/0

of adducts isolated from both in vivo and in vitro experiments with DNA. In particular the results presented in this paper concerning the diester **3** and the monoester **5** and in the preceding paper concerning the monoester **4** clarify the likely importance of the three types of ester in the initiation of oncogenesis.

Results

Synthesis of Monoester 5. In a preliminary communication[7] we described the preparation of the hydrochloride of the monoester **5** from the diacetate **3**. This preparation relies upon the preferential cleavage of the 4-acetoxyamino group in strong acid conditions. The optimum method of preparation of the monoacetate **5** requires the use of dry hydrogen chloride in chloroform. In aqueous solutions side reactions intervene, and the isolation of the hydrochloride of the monoester **5** is impossible due to its high reactivity. When the conversion of the diacetate **3** to the monoacetate **5** is carefully controlled in chloroform, good quality samples of the hydrochloride of the monoacetate **5** can be prepared routinely. This solid hydrochloride is rather unstable: on exposure to moisture decomposition is rapid. In the following studies freshly prepared samples were always used.

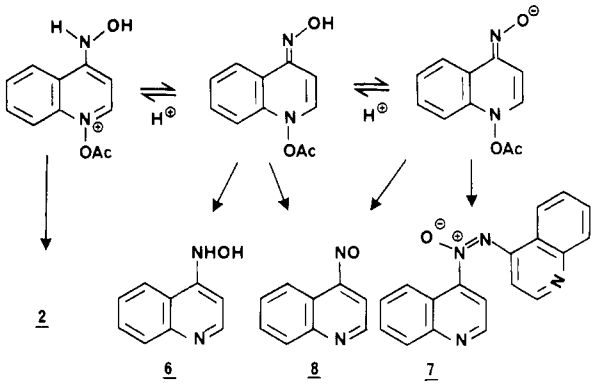
Although the monoacetate **5**, as the hydrochloride, is too unstable to permit satisfactory microanalysis, it has been thoroughly characterized spectroscopically. The mass spectral data clearly indicate a monoacetate structure, and the observation of the ester carbonyl (at 1825 cm^{-1}) indicates the presence of the acetoxyamino group. Acetylation of the monoacetate **5** affords the original diacetate **3**. As described below hydrolysis of the monoacetate **5** gives the dihydroxy derivative **2**, which is also obtained from the second monoacetate **4**. Two features of their ^1H NMR spectra permit the distinction between the two monoacetates. An oximino acetate, as in the ester **4**, is characterized by a signal of the methyl group at 2.10–2.20 ppm, whereas in an (hydroxylamino)acetate the corresponding signal is at 2.35 ppm. In the protonated esters a similar distinction is noted: the methyl signal in the oximino ester is observed at 2.35 ppm, but in the hydroxylamine ester it is observed at 2.55 ppm.

We have chosen to follow the chemistry of the monoacetate **5** by using HPLC analysis. However, there is a complication in the analysis of the monoacetate **5**. Under normal analytical conditions (see the Experimental Section) a single sharp peak is observed for the monoacetate **5**. However, this peak corresponds to 4-nitrosoquinoline (**8**). The decomposition of the monoacetate **5** on the stationary phase to give the nitroso compound **8** was easy to prove.

When the monoacetate **5** was stirred in a methanol–water mixture (pH 2.5) in the presence of the C-18 analytical phase, the nitroso compound **8** was rapidly formed as the only product. Isolation and characterization (see below) of the nitroso compound **8** implies that the HPLC analysis, in spite of the transformation of the monoacetate **5**, can safely be used to determine the monoacetate **5** quantitatively.

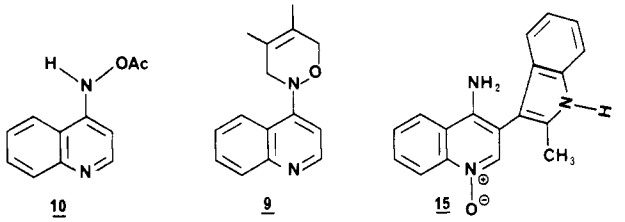
Product Studies. The full characterization of products from reactions of the monoacetate **5** is difficult. In most cases the products are very unstable and highly polar. Hence it has been necessary, in certain cases, to supplement spectroscopic characterizations with other data. Two strategies have been important. Highly polar hydroxy compounds have been acetylated, thus permitting a more complete characterization of the acetates. Secondly, careful use of HPLC analysis involving identifications by comparison with standard samples has facilitated structural identifications.

Scheme II



The hydrolysis of the monoacetate **5** has been examined under a variety of reaction conditions. In concentrated hydrochloric acid the monoacetate **5** is quantitatively (HPLC) converted to the dihydroxy derivative **2**. The latter compound has been described and characterized elsewhere.[5] In basic media the decomposition of the monoacetate **5** is extremely rapid and leads in the case of aqueous sodium hydroxide (1 N) to the formation of a red precipitate. The mass spectrum of this material suggested the possible formation of the azoxy compound **7**. In intermediate pH ranges the behavior of the monoacetate **5** is complex, but the results obtained in this pH range clarify the origin of the azoxy compound **7**, noted above.

In concentrated hydrochloric acid (6 N), although the dihydroxy compound **2** is the major product, the hydroxylamine **6** is observed as a minor product. In hydrochloric acid (1 N) this hydroxylamine **6** is a major product (>50%). Between pH 1 and 7 a new compound, the nitroso derivative **8** is observed, and at pH 5 this compound accounts for 80% of the products. This nitroso compound **8** has been reported earlier in our preliminary communication,[7] and it has been isolated, characterized spectroscopically (^1H NMR, UV, and IR), and transformed[8] by reaction with 2,3-dimethylbutadiene to the solid adduct **9**.



The hydroxylamine **6**, observed at pH 1, was shown by chromatography (HPLC and TLC) to be identical with a standard sample. The isolation of the hydroxylamine **6** was further confirmed by acetylation, which permitted the preparation of the more stable **10**, and comparison again with a standard sample. The diverse products obtained from the monoacetate **5** are shown in the Scheme II. We conclude that the azoxy derivative **7** probably originates from reaction of the nitroso derivative **8** with the hydroxylamine **6**.

(7) Demeunynck, M.; Lhomme, M. F.; Lhomme, J. *Tetrahedron Lett.* **1981**, *22*, 3189.

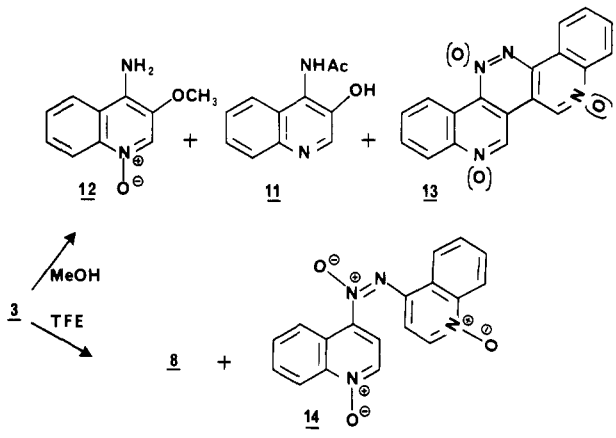
(8) Demeunynck, M.; Lhomme, M. F.; Lhomme, J. *J. Heterocycl. Chem.* **1984**, *21*, 501.

Table I. Kinetics of Hydrolysis of Diester 3 and Monoester 5

	[HCl]					
	12 N	6 N	3 N	2 N	1 N	10^{-1} N
$k_{25°}$ (s^{-1}) 5 × 10^4	2.09 ± 0.23	1.48 ± 0.03	3.33 ± 0.08	6.50 ± 0.36	14.61 ± 0.83	nd
r[a]	0.993	0.998	0.998	0.995	0.998	
$k_{25°}$ (s^{-1}) 3 × 10^4	12.03 ± 0.50	4.75 ± 0.10	7.16 ± 0.41	10.08 ± 0.28	12.19 ± 1.16	15.77 ± 0.28
r[a]	0.998	0.999	0.993	0.998	0.991	0.999

[a] "r" correlation coefficients for a linear treatment of concentration versus time data.

Scheme III



In a study of the hydrolysis of the diacetate **3** at pH 7, it was found that in the complex reaction mixture the ester **10** was obtained as a minor product. In order to establish more clearly the solvolytic behavior of the diacetate **3**, under neutral conditions, we have studied the solvolysis of the diacetate **3** in methanol, ethanol, and the more ionizing trifluoroethanol (Scheme III). In methanol two products, the amide **11** and the ether **12**, are obtained and have been fully characterized. In addition a mixture of dimers **13** is obtained. The relative yield of these dimers is determined by the concentration of the ester **3** in the reaction medium. At high concentrations the dimers **13** become the dominant products. The ether **12** has been described in a previous paper,[9] and the dimers **13** have been reported[10] by Japanese workers. The amide **11** is characterized on the basis of spectroscopic data (MS and ^{1}H NMR) (crucially by observation of two resonances at 9.73 and 9.86 ppm, which disappeared on addition of D_2O). In ethanol, the diacetate **3** analogously afforded the amide **11** in low yield. However, in trifluoroethanol containing water (3%), the diacetate **3** behaved differently. Although the amide **11** is observed as a minor product, the major products are the nitroso compound **8**, which was isolated and characterized by comparison with an authentic sample, and a dimeric product, of uncertain structure. Although this dimer was obtained in an impure state and has not been fully characterized, mass spectroscopic evidence suggested formation of the dimer **14**.

Some of the above products arise by attack of nucleophilic solvents in the solvolysis of the diacetate **3**. It was therefore of interest to study the possible reaction of the diacetate **3** with other nucleophiles and in particular because of the possible reaction with DNA bases, with reactive heterocyclic systems. We find that reaction of 2-methylindole with the diacetate **3** in methanol gives, by formation of a carbon–carbon bond, the *N*-oxide **15** (characterized by MS, ^{1}H NMR, and UV) in 16% yield. However, other products, e.g. the amide **11**, the ether **12**, and the dimers **13**, characteristic of the reaction of the diacetate **3** were also observed.

Spectral Analysis as a Function of pH. The behavior as acids and bases is a key feature of the chemistry of the esters **3–5**. In order to understand their properties it has been helpful to examine their UV spectra as a function of pH. The monoacetate **5** at pH 0.2 has the same spectrum as that in concentrated hydrochloric acid (12 N) (maxima at 230 and 342 nm). However between pH 0.2 and 1 a change in spectrum is observed (maxima at 242 and 350 nm). Above pH 1 the monoacetate **5** becomes too unstable to permit accurate spectra to be recorded. We conclude that below pH ~0.5 protonated monoacetate is present and above pH ~1 the neutral monoacetate **5** is the dominant species.

Similarly the protonation of the diacetate **3** is readily recognized. In strong acid (pH <1) two maxima are observed at 238 and 335 nm, but at pH 3 new bands at 254 and 348 nm appear. Again we consider that below pH 1 the diacetate **3** is protonated. Analysis of the spectra indicates that the pK_a of the conjugate acid of the diacetate **3** is between pH 1.5 and 2.

Kinetic Studies. The hydrolysis of the monoacetate **5** was studied in aqueous acid media. Although the loss of the ester was briefly examined in the less acidic media (pH >1) we are unable to report quantitative results in this region due to the exceptionally fast reaction of the ester. In the more acidic solutions first-order kinetics were observed (>90% of reaction) for the loss of the monoacetate **5** (HPLC). The first-order rate constants for both loss of the monoacetate **5** and the diacetate **3** are shown in Table I.

Discussion

The solvolytic behavior of the monoacetate **5** is determined by the pH of the medium. The kinetic results shown in Table I establish the two important regions of acidity. In highly acidic media the protonation of the monoacetate **5** "protects" the compound. Hence at this activity the only product, the dihydroxy compound **2**, is formed by attack at the carbonyl function. In solutions of less acidity other reactions are observed, which eventually compete to the exclusion of the formation of the dihydroxy compound **2**. The observation of a rate increase in the loss of the monoacetate **5** as the pH is raised up to pH 1 is explained by these other processes.

In the second region of pH above pH 1 the monoester **5** affords a complex product mixture including the nitroso compound **8**, the hydroxylamine **6**, and diverse dimeric products. In these more neutral conditions products arise by cleavage of the nitrogen–oxygen bond. Although their formation might be via a homolytic cleavage, the alternative heterolytic cleavage facilitated by the oximino oxygen participation appears likely. Such a cleavage accounts for both the elimination product, the nitroso derivative **8**, and the reduction product, the hydroxylamine **6**. With

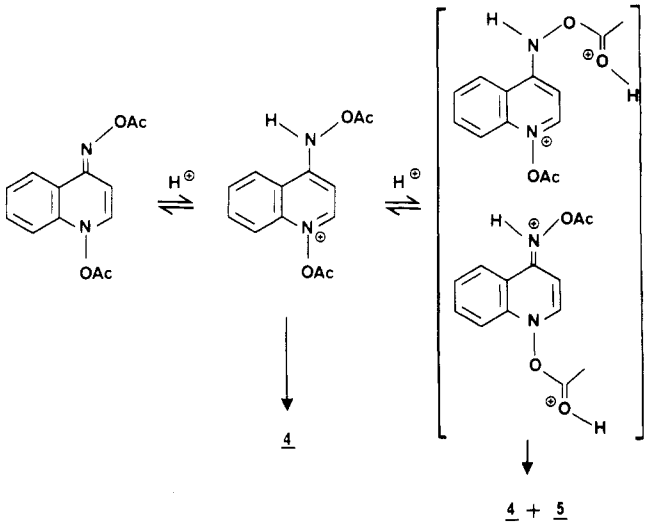
(9) Demeunynck, M.; Tohme, N.; Lhomme, M. F.; Mellor, J. M.; Lhomme, J. *J. Am. Chem. Soc.* **1986**, *108*, 3539.

(10) (a) Kosuge, T.; Zenda, H.; Yokota, M.; Sawanishi, H.; Suzuki, Y. *Chem. Pharm. Bull. (Tokyo)* **1969**, *17*, 2181. (b) Kosuge, T.; Zenda, H.; Sawanishi, H. *Ibid.* **1969**, *17*, 2389. (c) Kosuge, T.; Yokota, M. *Yakugaku Zasshi* **1965**, *85*, 69.

Table II. Acidic Hydrolysis of Diester 3: Influence of the Acid Concentration on the Yield of Reaction Products, Monoesters 4 and 5

	[HCl]				
	12 N	9 N	6 N	3 N	1 N
4, %	0	5	16	48	85
5, %	46	35	15	3	0

Scheme IV



derivatives of purine *N*-oxides analogous results and interpretations have been reported.[11] The oxygen participation in the case of the monoester **5** would be particularly facilitated in basic media as a result of ionization.

The study of the effect of pH upon the spectrum of the monoester **5** confirms the above analysis. In the pH range between concentrated hydrochloric acid (12 N) and pH ~1 the invariance of the spectrum indicates the protonation of the ester. It is this protonation that retards hydrolysis. Above pH 1 the neutral monoester **5**, characterized by a different spectrum from the protonated species, is readily hydrolyzed via nitrogen–oxygen cleavage.

The above results lead to a better appreciation of the chemistry of the diacetate **3**. As shown in Table I, the diacetate **3** shows a more complex pattern of reactivity than the monoacetate **5**. The rate of hydrolysis is modestly affected by pH as the acidity decreases from 6 N hydrochloric acid so the rate of hydrolysis increases. At pH 1–2 a maximum rate is observed, and further increase of pH leads to a decrease in the rate of loss of the diacetate **3**. A brief summary of the products from the diacetate **3** is given in Table II and has been discussed more fully elsewhere.[6] The likely mechanisms of hydrolysis of the diacetate **3** are shown in Scheme IV. In strong acid (12 N hydrochloric acid) the half-life of the diacetate **3** ($t_{1/2}$ = 9 min) is similar to that of the monoacetate **4** ($t_{1/2}$ = 5 min), whereas at this pH the monoacetate **5** is more stable ($t_{1/2}$ = 55 min) (see Figure 2). These results show that the cleavage of the acetoxyimino group in the protonated diacetate **3** and the monoacetate **4** is scarcely influenced by the nature of the 1-substituent. In addition these results explain the selective monocleavage of the diacetate **3** in strong acid.

In contrast, in weaker acid (1 N hydrochloric acid) while the diacetate **3** ($t_{1/2}$ = 8 min) and the monoacetate **5** ($t_{1/2}$ = 8 min) have a comparable reactivity, it is now the other monoacetate **4** ($t_{1/2}$ = 113 min) that is the more stable at this pH. Again the selectivity of hydrolysis of the diacetate **3** at pH 1 is readily understood. Whatever the nature of the protonated species (likely species are shown in Scheme IV), the rate of hydrolysis of the 1-acetoxy function is not influenced by the nature of substitution at the 4-position.

(11) Parham, J. C.; Templeton, M. A. *J. Org. Chem.* **1982**, *47*, 652 and preceding papers.

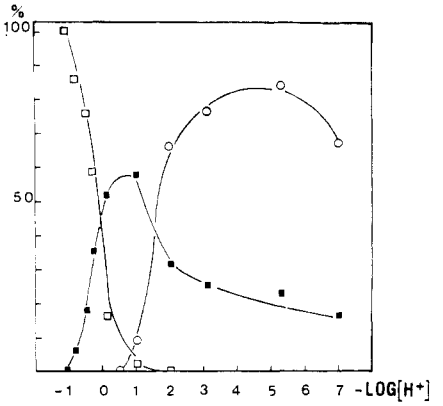


Figure 1. Solvolysis of **5**. Effect of pH on the yields of identified products: (□), dihydroxylated derivative **2** (hydrolysis compound); (■), hydroxyaminoquinoline **6** (reduction compound); (○), 4-nitrosoquinoline **8** (elimination compound).

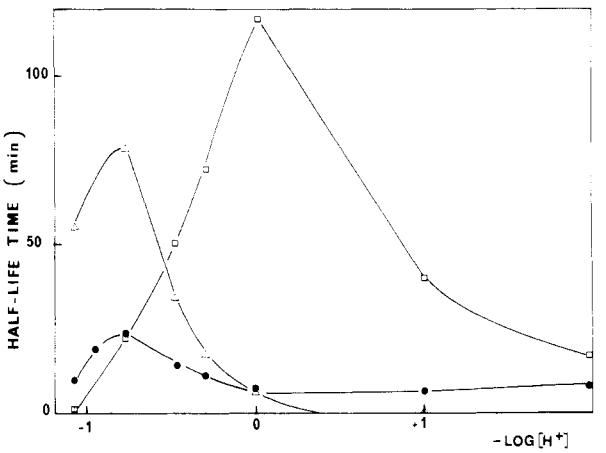


Figure 2. Comparative solvolysis in acidic medium, influence of the acid concentration on the rate of hydrolysis: (●), diester **3**; (□), monoester **4**; (△), monoester **5**.

Although an understanding of the hydrolytic behavior of the monoacetates **4** and **5** and the diacetate **3** is crucial to an understanding of the possible biological role of the esters of the dihydroxy compound **2**, a further important consideration is the possible mode of action of these esters with possible nucleophiles such as DNA bases. The 1-ester function in the diacetate **3** reacts selectively via oxygen–acyl cleavage to give the monoacetate **4** in nonnucleophilic solvents such as dimethyl sulfoxide in the presence of added nucleophiles, e.g. piperidine or thiophenol. Under these reaction conditions, where the product is stable, the monoacetate is readily prepared. In methanol the diacetate **3** gave the 3-methoxy derivative **12**. Similarly in methanol, in the presence of 2-methylindole, acting as a nucleophile, reaction affords an adduct via reaction of the diacetate **3** at the 3-position. Hence there is a close parallel in the chemistry of diacetate **3** and the monoacetate **4**, as products can be formed via nitrenium ion intermediates with reaction of external nucleophiles at the 3-position. In contrast under similar reaction conditions the monoacetate **5** reacting via the neutral non protonated species gives products via nitrogen–oxygen cleavage. Such cleavage in the monoacetate **5** is facilitated by the oxime function. Yet even in the case of the diacetate **3**, the

observation that the nitroso compound 8 is formed in trifluorethanol suggests that in these highly ionizing conditions there is a reaction pathway involving direct loss of the 1-ester function by nitrogen–oxygen cleavage. Assistance by the second ester function in the diacetate 3 is indicated by observation of formation of the amide 11 as a product under these conditions. A further pathway for reaction of the diacetate 3 with nucleophiles has been recognized. In trifluoroethanol, conditions of low nucleophilicity, in the presence of adenine derivatives the pathway implies an initial Michael type addition.[12]

Studies with nucleosides have shown that by their reaction with derivatives of 4-nitroquinoline *N*-oxide products can be isolated. Four such adducts have been described. All correspond to an initial attack at the 3-position[13] or at the nitrogen group[14] substituted at the 4-position. This attack at the 3-position is exactly the result observed in reaction of nucleophiles with our model esters. In the case of the monoacetate 4 such an attack is attributed to the intermediacy of a nitrenium ion. Similarly in reaction of the diacetate 3 with 2-methylindole to give the adduct 15, we postulate attack at the 3-position. Hence both the model monoacetate 4 and the diacetate 3 react via nitrenium ion intermediates at the 3-position. We conclude that those products, derived from reaction of derivatives of 4-nitroquinoline *N*-oxide with nucleosides, are formed by a similar pathway. In contrast it appears that esters analogous to the monoacetate 5 have a less significant role. Not only is this type of ester unlikely to be formed from a diester analogous to 3 at a biological pH but if formed it would react rapidly at this pH by nitrogen–oxygen cleavage.

It is possible to extrapolate our kinetic data (see Table I) to understand better the likely biological role of the three types of ester. That type analogous to 5 is considered to be unimportant, for the above reasons. An extrapolation of the kinetic data for the monoacetate 4 using the Grunwald Winstein plot[5] indicates a rate constant for reaction in water (pH 7, at 25 °C) of $k = 9 \times 10^{-3} s^{-1}$ ($t_{1/2} \simeq 1$ min). This implies a very short half-life for a possible monoester ultimate metabolite of this type. For comparison the half-life of the ultimate metabolite of the well-known carcinogen aminofluorene is $t_{1/2} = 2$ h. Hence the biological role of monoesters analogous to the acetate 4 is likely to be complicated by the instability of such esters. Side reactions will limit the ability of such esters to be transported intact from a remote site to an active site on DNA.

Experimental Section

General Procedures. ^1H NMR spectra were recorded on a Bruker WP 60 (60 MHz). Chemical shifts are reported in ppm (δ) relative to hexamethyldisiloxane as internal standard. Mass spectra were recorded on a Riber-Mag 10-10 and a Varian MAT 311 spectrometer. UV spectra were recorded on a Beckman DB-GT and a Perkin Lambda 15 spectrometer. IR spectra were recorded on a Perkin-Elmer 237 instrument. Melting points are uncorrected. Reversed-phase HPLC was performed with a μ-Bondapak C18 analytical column (Waters Associates) equipped with a Model 660 solvent programmer and two M-6000 pumps (Waters Associates). The effluent was analyzed by a dual wavelength detector (254, 365 nm). A linear gradient of solvents was used from 10 to 100% methanol in water, pH 2.5 (phosphoric acid), during 10 min with a 2 mL/min flow rate.

Preparation of Starting Materials and Reference Compounds. The syntheses of 1-hydroxy-4-(hydroxyimino)-1,4-dihydroquinoline (2), 1-acetoxy-4-(acetoxyimino)-1,4-dihydroquinoline (3), and 4-(hydroxyamino)quinoline (6) are described on the preceding paper.[5]

1-Acetoxy-4-(hydroxyimino)-1,4-dihydroquinoline hydrochloride (5) was prepared as previously described.[7] The diester 3 (0.2 g, 0.77 mmol) was dissolved in dry chloroform (100 mL), and a stream of dry hydrogen chloride was bubbled through the solution during 5 min. The solution was left for 1 week at −20 °C, after which white crystals had deposited. The solid was filtered and carefully washed with cold chloroform and anhydrous ether to afford monoester 5 as the hydrochloride in 76% yield: mp 118–118.5 °C; UV max λ nm (MeOH) 242 (ϵ = 21 000), 354 (ϵ = 11 000); IR (KBr) 3000, 1825, 1610, 1560, 1405, 1365, 1140, 1115, 1030, 995, 810, 765, and 755 cm^{-1}; ^1H NMR (CD_3OD, DCl) δ 8.70 (d, 1 H, J = 8 Hz, H-2), 8.40 (m, 1 H), 7.50–8.00 (m, 3 H), 7.05 (d, 1 H, J = 8 Hz, H-3), 2.50 (s, 3 H, $COCH_3$); MS, m/e (relative intensity) 219 ($M^+ - 1 - HCl$, 4), 202 (0.2), 174 (3), 160 (31), 144 (58), 129 (49), 128 (100), 117 (34).

4-Nitrosoquinoline (8) was prepared in 60% yield by oxidation of the corresponding hydroxylamine 6 with silver carbonate on Celite as previously described:[8] mp 82–83 °C; UV max λ nm ($CHCl_3$) 242 (ϵ = 20 140), 364 (ϵ = 8400); ^1H NMR ($CDCl_3$) δ 9.70 (m, 1 H), 9.10 (d, 1 H, J = 4.2 Hz, H-2), 7.50–8.50 (m, 3 H), 6.10 (d, 1 H, J = 4.2 Hz, H-3).

4,5-Dimethyl-3,6-dihydro-1-(4-quinolyl)-1,2-oxazine (9) was synthesized as previously described[8] by reaction of 2,3-dimethylbutadiene with the nitrosoquinoline 8 in chloroform in 70% yield: mp 100–101 °C; ^1H NMR ($CDCl_3$) δ 8.75 (d, 1 H, J = 4.9 Hz, H-2), 7.90–8.10 (m, 2 H), 7.40–7.70 (m, 2 H), 7.15 (d, 1 H, J = 4.9 Hz, H-3), 4.35 (s, 2 H, OCH_2), 3.70 (s, 2 H, $N\text{-}CH_2$), 1.65 (s, 6 H, $2CH_3$); MS, m/e (relative intensity) 240 (M^+, 66), 222 (74), 221 (58), 207 (38), 206 (15), 195 (6), 158 (27), 144 (17), 129 (20). Anal. Calcd for $C_{15}H_{16}N_2O$: C, 74.97; H, 6.71; N, 11.65. Found: C, 74.84; H, 6.97; N, 11.56.

Methodology for Kinetic Experiments. Hydrolyses of the monoester 5 were studied in various aqueous media including aqueous hydrochloric acid at pH 2 and below, citrate phosphate buffer between pH 3 and 7, and aqueous sodium hydroxide.

All kinetics were performed with 4×10^{-3} M aqueous solutions. Samples of the monoester (5 mg) were dissolved in the appropriate solvent (5 mL), and the reactions were allowed to proceed at 25 ± 0.5 °C in the dark. The disappearance of the starting material and the formation of reaction products were monitored as a function of time by removing aliquots (5 μL) and analyzing them by HPLC. Peak heights were used to determine pseudo-first-order rate constants. All solvolyses were followed up to 95% conversion, and duplicate experiments were run. Average first-order-rate constants were reported with average deviation of ±5%.

HPLC Analysis of the Monoester 5. This compound was routinely analyzed by HPLC. The chromatogram shows one peak, which corresponds to 4-nitrosoquinoline (8) (retention time and ratio between absorption intensities at the two different wavelengths). The nitroso derivative is quantitatively formed in our HPLC conditions as shown by control experiments: a sample (0.5 mL) of a methanolic solution (4×10^{-3} M) of the monoester was placed on a μ-Bondapak C_{18} analytical phase (Sep-Pak C_{18} Cartridge, Waters Associates). The product absorbed on the solid phase was extracted by using successively a methanol–water mixture (10:90, 2 mL) and then pure methanol (2 mL); the resulting method extract was analyzed by HPLC and TLC in different systems and had retention time[5] and R_f values identical to those observed for the nitrosoquinoline. The methanol was then evaporated to dryness, and the residue was diluted with chloroform and analyzed by UV spectrometry. The UV spectrum shows the characteristic absorption of the nitroso compound: UV max λ nm ($CHCl_3$) 242, 364.

To distinguish the monoester 5 from its decomposition product 8 we have carried out further reactions. Extraction of the nitrosoquinoline by methylene chloride as soon as it had formed was followed by the trapping of the nitroso compound 8 with a diene, and immediate formation of a dihydrooxazine 9 occurred (see below).

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Due to the lack of stability of the reactions products in aqueous solutions, they were identified by comparison of their HPLC and TLC characteristics with those of authentic samples. Yields were obtained from calibration experiments by HPLC analysis. To confirm the structure of the reaction products some control experiments have been made.

Reactions in Acidic Medium (Methanol–7 N Hydrochloric Acid): Formation of a Mixture of the Dihydro Compound 2 and (Hydroxyamino)quinoline 6. The reaction was followed at the same time by HPLC and ^{1}H NMR analyses. The HPLC analysis indicated that two products **2** and **6** were formed; signals corresponding to the two compounds were found at 7.1 (H-3) and 8.3 (H-2) ppm for the (hydroxyamino)quinoline **6** and at 7.0 (H-3) and 8.6 (H-2) ppm for the dihydro derivative **2**. We have checked that a mixture of authentic samples of the two compounds give exactly the same ^{1}H NMR spectrum under the same solvent conditions.

Formation of 1-Hydroxy-4-(hydroxyimino)-1,4-dihydroquinoline (2) in 12 N Hydrochloric Acid: Acetylation to Diacetate 3. A solution of the monoester **3** (4×10^{-3} M) in 12 N hydrochloric acid was allowed to react for 3 h. The solvent was evaporated to dryness, and the residue was immediately dissolved in acetic anhydride (2 mL). The solution, thus obtained, was analyzed by HPLC and TLC. The only product formed was identified as the diester **3**.

Formation of 4-(Hydroxyamino)quinoline (6) in 1 N Hydrochloric Acid. The reaction mixture of **5** in 1 N hydrochloric acid was evaporated under reduced pressure after 1 h. Treatment of the residue with acetic anhydride (2 mL) and dimethylformamide (2 mL) afforded the monoacetate **10** as shown by TLC and HPLC. The yield based on the initial weight of monoacetate **5** was 42%.

Formation of 4-Nitrosoquinoline (8) in Aqueous Solutions at pH 2: Reaction with the 2,3-Dimethylbutadiene. The monoester **5** (0.02 g, 0.07 mmol) was dissolved in the buffer (20 mL, pH 3, 5, and 7). Ether was immediately added to extract the nitrosoquinoline **8**, which was identified by UV, HPLC, and TLC comparison with an authentic sample. A large excess of 2,3-dimethylbutadiene (0.1 mL) was then added to the organic fraction. After 15 min, the solvent was evaporated, and the product **9** was compared (^{1}H NMR, MS) with the authentic sample.

Formation of Azoxyquinoline 7 in Basic Solution. The monoester **5** (0.02 g, 0.07 mmol) was dissolved in 1 N aqueous sodium hydroxide (2 mL) with vigorous stirring. After 1 h the deposited red solid was filtered and analyzed by MS: MS, *m/e* (relative intensity) 300 (M^+, 35), 284 ($M^+ - 16$, 22), 282 (39), 271 (33), 255 (8), 149 (34), 144 (28), 142 (40), 141 (33), 128 (100).

Reaction of Diacetate 3 with Methanol. Preparation of Compounds 11–13. Diacetate **3** (0.1 g, 0.38 mmol) was dissolved in methanol (200 mL). The reaction was run at room temperature in the dark and under nitrogen. After 3 days a solid was deposited: it was removed by filtration (0.016 g, 26%) and identified by mass spectroscopy as a mixture of [3,4-C; 5,6-C'] pyridazinoquinoline mono-, di-, or trioxides (**13**):[10] HMRS, *m/e* 330 (M^+), 314, 282, 257, 157, 141, 115.

The solvent was removed in vacuo, and the residue was chromatographed on a silica gel column with ethyl acetate–ethanol as eluant (96:4). This yielded the following two compounds in the order of elution:

(a) 4-(Acetylamino)-3-hydroxyquinoline (11) (0.009 g, 11%): mp 249–250 °C; ^{1}H NMR (DMSO-d_6, 270 MHz) δ 9.86 (s, 1 H), 9.73 (s, 1 H), 8.56 (s, 1 H), 7.86 (d, 1 H), 7.77 (d, 1 H), 7.46 (m, 2 H), 2.11 (s, 3 H); HRMS calcd for $C_{11}H_{10}N_2O_2$ 202.0742, found 202.0749; MS, *m/e* 202 (M^+), 184, 160, 144, 131.

(b) 4-Amino-3-methoxyquinoline 1-oxide (12) (0.027 g, 32%): mp 205 °C (lit.[15] mp 226 °C); UV max λ nm ($CHCl_3$) 385 (ϵ = 3400), 360 (ϵ = 5000), 330 (ϵ = 6730), 285 (ϵ = 8840); IR ($CHCl_3$) 3410, 2830, 1625, 1590, 1340 cm^{-1}; ^{1}H NMR (DMSO-d_6, 270 MHz) δ 8.33 (s, 1 H, H-2), 8.31 (d, 1 H, J = 9 Hz, H-8), 8.15 (d, 1 H, J = 9 Hz, H-5), 7.36–7.22 (m, 2 H), 6.33 (s, 2 H, NH_2), 3.83 (s, 3 H, OCH_3); HRMS calcd for $C_{10}H_{10}N_2O_2$ 190.0724, found 190.0749; MS, *m/e* 190 (M^+), 174, 159, 144, 131.

Reaction of Diacetate 3 with Trifluoroethanol. Diester **3** (0.05 g, 0.19 mmol) was dissolved in trifluoroethanol (15 mL). The solution was kept at room temperature, in the dark, under nitrogen. Dimeric azoxy compounds **14** were separated by filtration (0.008 g, 26%) after 24 h: ^{1}H NMR (DMSO-d_6, 270 MHz) δ 9.03 (d, 1 H), 8.78–8.48 (m, 6 H) 8.25 (d, 1 H), 7.97–7.75 (m, 4 H); HRMS calcd for $C_{18}H_{12}N_4O_3$ 332.0909, found 332.0916; MS, *m/e* 332 (M^+), 316, 300, 284, 271, 255, 158, 144.

The liquid residue, after removal of the solvent in vacuo, was chromatographed on silica gel column. This yielded the following two compounds **8** and **11** in order of elution:

(a) 4-Nitrosoquinoline (8). Obtained with chloroform as eluant (0.011 g, 30%), it is identified by comparison with a synthetic sample.

(b) 4-(Acetylamino)-3-hydroxyquinoline (11): isolated with an ethyl acetate–ethanol mixture (96:4) (0.002 g, 3%).

Reaction of Diacetate 3 with 2-Methylindole in Methanol. Preparation of Compounds 11, 12, and 15. Diacetate **3** (0.1 g, 0.38 mmol) and 2-methylindole (0.15 g, 1.15 mmol) were dissolved in methanol (200 mL). The reaction was run at room temperature, under nitrogen, and in the dark. After 3 days the dimeric compounds as previously described **13** were eliminated by filtration (30%). After removal of the solvent the crude residue was chromatographed on a silica gel column with an ethyl acetate–ethanol (96:4) mixture as eluant. This yielded the following three compounds in order of elution:

(a) 4-(Acetylamino)-3-hydroxyquinoline (11) (0.010 g, 12%).

(b) 4-Amino-3-methoxyquinoline 1-oxide (12) (0.025 g, 30%).

(c) 4-Amino-3-(2-methylindol-3-yl)quinoline 1-oxide (15) (0.025 g, 16%): mp 258–260 °C; UV max λ nm (EtOH) 380 (ϵ = 7225), 320 (ϵ = 8265), 284 (ϵ = 11 600), 282 (ϵ = 13 500), 262 (ϵ = 19 000); IR ($CHCl_3$) 3470, 2870, 1600 cm^{-1}; ^{1}H NMR (DMSO-d_6, 270 MHz) δ 11.30 (s, 1 H), 8.46 (d, 1 H, J = 9 Hz, H-8), 8.28 (d, 1 H, J = 9 Hz, H-5), 8.02 (s, 1 H, H-2), 7.75–7.50 (2 m, 2 H, H-6, H-7), 7.28 (d, 1 H, J = 7.5 Hz, H-4 Ind), 7.11 (d, 1 H, J = 7.5 Hz, H-7 Ind), 7.05–6.83 (2 m, 2 H), 6.11 (s, 2 H, NH_2), 2.24 (s, 3 H, H-3 Ind); HRMS calcd for $C_{18}H_{15}N_3O$ 289.1215, found 289.1226; MS, *m/e* 289 (M^+), 273, 257, 237, 181, 128.

Reaction of Monoacetate 4 with 2-Methylindole. Preparation of Compound 15. Diester **3** (0.011 g, 0.0042 mmol) was dissolved in dimethyl sulfoxide (1 mL); piperidine (0.004 mL, 0.042 mmol) was added to the solution to give the monoester **4** as indicated by HPLC analysis performed after 15-min reaction. 2-Methylindole (0.055 g, 0.42 mmol) was added to the mixture. After 24 h in the dark, at room temperature, and under nitrogen, the solvent was removed in vacuo. The residue was treated by preparative thin-layer chromatography on silica gel. Elution by ethyl acetate–methanol (96:4) gave compound **15** (0.005 g, 32%).

4-Amino-3-(2-methylindol-3-yl)quinoline 1-oxide (15) is identified by comparison with the compound isolated in the reaction of diester **3** with 2-methylindole.

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