of benzene, with $Co_2(CO)_8$ as catalyst precursor. Reactant ratios and reaction mediums are given in the footnotes of Table III.

Synthesis of Benzolactams and Benzolactones (Table IV). Carbonylations were conducted as described above in a two-phase system: C_6H_6/NaOH(aq) (reactant ratios are given in the footnotes of Table IV). At the end of the reaction, lactams were present in the organic phase which was washed several times with water and dried over $MgSO_4$. On the contrary, isolation of lactones was achieved by acidification of the aqueous phase followed by a classical workup and drying over $MgSO_4$. All cyclized compounds were purified by column chromatography on a short silica gel column or by recrystallization. Satisfactory analytical data (±0.4% for C, H, and N) were obtained for all new compounds listed in Table IV. They were characterized by comparison with literature data (melting point, IR, NMR) or on the basis of their spectroscopic properties (IR, NMR).

Benzolactam 2: colorless oil; IR (film) ν_{max} 3250, 1665 (s), 1605, 1575 cm^{-1}; NMR ($CDCl_3$) δ 2.90 (t, 2 H), 3.3–3.7 (m, 2 H), 7.0–7.6 (m, 3 H, aromatic), 7.7–8.4 (m, 2 H, aromatic and NH).

Benzolactam 4: mp 151 °C (lit.[27] mp 150 °C); IR (Nujol) ν_{max} 3220, 1685 cm^{-1}; NMR (C_3D_6O) δ 4.6 (s, 2 H), 7.5–8.1 (m, 5 H, aromatic and NH).

Benzolactam 6: colorless liquid; IR (film) ν_{max} 3220, 1670, 1605, 1575 cm^{-1}; NMR ($CDCl_3$) δ 0.9 (t, 3 H), 1.3–2.0 (m, 2 H), 2.5–3.0 (m, 1 H), 3.1–3.9 (m, 2 H), 7.1–7.9 (m, 3 H, aromatic), 7.8–8.3 (m, 2 H, aromatic + NH).

Benzolactam 8: mp 91 °C (lit.[21] mp 90–91 °C); IR (Nujol) ν_{max} 1680, 1620, 1600 cm^{-1}; NMR ($CDCl_3$) δ 4.25 (s, 2 H), 4.80 (s, 2 H), 7.1–7.6 (m, 8 H, aromatic), 7.7–8.1 (m, 1 H, aromatic).

Benzolactone 10: IR (film) 1720 cm^{-1} (large); NMR ($CDCl_3$) δ 3.0 (t, 2 H), 4.5 (t, 2 H), 7.1–7.8 (m, 3 H, aromatic), 7.8–8.3 (1 H, aromatic).

Benzolactone 12: mp 72–73 °C (petroleum ether); (lit.[28] mp 73 °C); IR ($CDCl_3$) ν_{max} 1768 cm^{-1}; NMR ($CDCl_3$) δ 5.35 (s, 2 H), 7.4–7.8 (m, 3 H, aromatic), 7.8–8.2 (m, 1 H, aromatic).

Benzolactone 14: colorless liquid; IR (film) ν_{max} 1730 cm^{-1}; NMR ($CDCl_3$) δ 1.0 (t, 3 H), 1.5–2.1 (m, 2 H), 2.6–3.1 (m, 1 H), 4.5 (d, 2 H), 7.2–7.8 (m, 3 H, aromatic), 8.0–8.4 (m, 1 H, aromatic).

Carbonylation of Vinyl Halides (Table V). Carbonylation of vinyl halides was carried out in a two-phase system [C_6H_6/NaOH(aq)] by using the general procedure described above for aryl halides. Reactant ratios are given in the footnotes of Table V. All isolated acids were identified by comparison (IR, NMR) with authentic samples, either commercial or prepared by standard methods.

Acknowledgment. This work was supported by the Société Nationale Elf Aquitaine (Production) and by the Centre National de la Recherche Scientifique (France) both of which are gratefully acknowledged. Both reviewers are also acknowledged for their fruitful comments.

Registry No. 1, 65185-58-2; 2, 1196-38-9; 3, 3959-05-5; 4, 480-91-1; 5, 67932-62-1; 6, 55150-61-3; 7, 65185-56-0; 8, 13380-32-0; 9, 1074-16-4; 10, 4702-34-5; 11, 18982-54-2; 12, 87-41-2; 13, 85029-25-0; 14, 85029-26-1; bromobenzene, 108-86-1; chlorobenzene, 108-90-7; iodobenzene, 591-50-4; 4-bromotoluene, 106-38-7; 2-bromotoluene, 95-46-5; 4-bromoanisole, 104-92-7; 2-bromoanisole, 578-57-4; 4-bromofluorobenzene, 460-00-4; 4-bromochlorobenzene, 106-39-8; 4-bromoacetophenone, 99-90-1; 4-bromonitrobenzene, 586-78-7; ethyl 4-bromobenzoate, 5798-75-4; (2-bromophenyl)acetamide, 65999-53-3; 1-bromonaphthalene, 90-11-9; 2-bromonaphthalene, 580-13-2; 4-chlorophenol, 106-48-9; 4-chlorobenzoic acid, 74-11-3; 4-chlorobenzeneacetic acid, 1878-66-6; 1-bromocyclooctene, 4103-11-1; 1-bromocyclohexene, 2044-08-8; 1-chlorocyclohexene, 930-66-5; 3,3-dimethyl-2-chlorobut-1-ene, 27843-27-2; (*E*)-β-bromostyrene, 588-72-7; (*Z*)-β-bromostyrene, 588-73-8; 4-bromophenol, 106-41-2; benzoic acid, 65-85-0; 4-methylbenzoic acid, 99-94-5; 2-methylbenzoic acid, 118-90-1; 4-methoxybenzoic acid, 100-09-4; 2-methoxybenzoic acid, 579-75-9; 4-fluorobenzoic acid, 456-22-4; 4-acetylbenzoic acid, 586-89-0; 4-hydroxybenzoic acid, 99-96-7; 4-nitrobenzoic acid, 62-23-7; terephthalic acid, 100-21-0; (2-carboxyphenyl)acetic acid, 89-51-0; 1-naphthoic acid, 86-55-5; 2-naphthoic acid, 93-09-4; 1-cyclooctenecarboxylic acid, 4103-09-7; 1-cyclohexenecarboxylic acid, 636-82-8; 2-*tert*-butylacrylic acid, 4423-82-9; *trans*-cinnamic acid, 140-10-3; *cis*-cinnamic acid, 102-94-3; dicobaltoctacarbonyl, 10210-68-1; tetrabutylammonium bromide, 1643-19-2.

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(28) W. Hessert, *Chem. Ber.*, **10**, 1445 (1881).

Solvolytic Behavior of 1-Acetoxy-4-(acetoxyimino)-1,4-dihydroquinoline, a Model for the Activated Form of the Carcinogenic 4-Nitroquinoline *N*-Oxide†

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The solvolytic behavior of the title compound **3**, a model for the activated form of the bifunctional carcinogenic 4-nitroquinoline 1-oxide (**1**) has been explored to find conditions for selective deacylation to the corresponding monoacetyl derivatives **4** and **5**. At pH 7, in a water–methanol mixture, the solvolysis is rapid ($1/2\ \tau$ = 6 min), quite unselective, and leads to a complex mixture. At basic pH, the 1-acetyl group is selectively removed to give the 4-monoacetyl derivative **4**, which immediately undergoes further reaction. Under highly acidic conditions, both monoacyl compounds **4** and **5** are formed. However, due to their different reactivity, **5** can be separated from **4** as its crystalline hydrochloride.

4-Nitroquinoline 1-oxide (**1**, 4-NQO) is a powerful carcinogenic compound whose mode of action at the molecular level is not fully understood.[1] A number of biological studies have led to the conclusion that the first activation step in vivo is the reduction to the (hydroxyamino)-quinoline 1-oxide **2**[2] (which exists mainly as its hydroxyimino tautomer **2b**).[3] This compound is subsequently converted to a metabolite(s) which is (are) able to bind covalently to nucleophilic sites of nucleic acid bases. Diacetate **3** and monoacetate **4** have been proposed as

†Dedicated to Professor Edgar Lederer on the occasion of his 75th birthday.

(1) For a recent review, see: Sugimura, T. "Carcinogenesis"; Raven Press: New York, 1981; Vol. 6.
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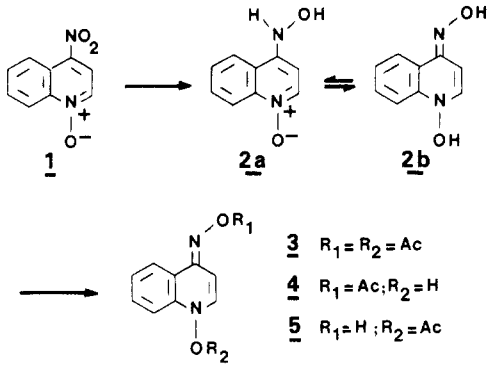
(3) Kawazoe, Y.; Ogawa, O.; Huang, G. F. *Tetrahedron* **1980**, *36*, 2933.

0022-3263/83/1948-1171$01.50/0

Scheme I



models for these final reactive species (Scheme I).[4,5] Indeed, the in vitro reaction of diacetate **3** with DNA and nucleosides has been shown to lead to minute amounts of products of addition to the bases.[6] One adduct with deoxyguanosine has recently been isolated and fully characterized.[7] This compound is also obtained when the monoacetyl derivative **4** (produced in situ) is reacted with the nucleoside.[7] It corresponds to one of the numerous products formed in the in vivo reaction with DNA.[8,9]

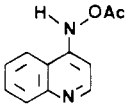
A clear understanding of the reactions involved in the modifications of DNA by the carcinogen is made difficult by the extreme reactivity of the postulated metabolites and by the number of reaction pathways which are possible. Even when a product of reaction between diacetyl derivative **3** and DNA or a nucleoside can be isolated, the yield is exceedingly low.[7] In addition, a number of other products may well result from reaction with partially hydrolyzed substrates such as the monoacetyl compounds **4** and **5** or with unknown byproducts. The chemistry of such compounds is still largely unknown.

The synthesis of the model diacyl derivative **3** has been reported,[4,5] but little is known about its chemical properties except that it is exceedingly reactive,[10] being sensitive to heat, oxygen, and light. A detailed and recent study by the pioneers in the field Kawazoe et al., has shown that it is partially deacylated to **4** when treated with ammonia or dithiothreitol in Me_2SO.[3] However, **4** is so reactive that it could not be isolated from the medium, which is a considerable limit to the study of its chemical reactivity with DNA and nucleosides. We have studied the hydrolysis of the diacetyl compound **3** over a wide range of pH and now report the conditions under which the individual monoacetates **4** and **5**,[11] both of which may be considered as activated forms of the metabolite **2**, can be obtained.

Results

1-Acetoxy-4-(acetoxyimino)-1,4-dihydroquinoline (**3**) was prepared according to Kawazoe et al. by acetylation of 4-HAQO (**2**),[4] the reduction product of 4-nitroquinoline 1-oxide.[5] Its behavior was studied in water and methanol–water solutions at different pH values.

Hydrolysis in Neutral Conditions: H_2O–CH_3OH. In a water–methanol (1:1) solution maintained at pH 7 by using a phosphate buffer, the transformation of **3** at 25 °C is rapid as shown by the HPLC analysis performed at different time intervals. Pseudo-first-order kinetics are observed with a half-life of 6min. The reaction product is exceedingly complex as more than 15 peaks are shown on the chromatogram. These were not identified except for one which corresponds to the already described (hydroxyamino)quinoline acetate **6**, resulting from an N_1–O cleavage.[12]



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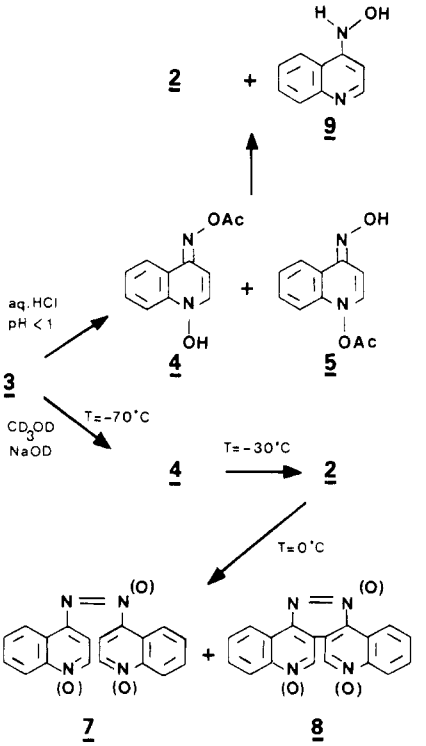
Hydrolysis under Basis Conditions: NaOH–H_2O–CH_3OH. Addition of aqueous sodium hydroxide (1 N NaOH) to a methanolic solution of **3** leads to the immediate appearance of an orange color and precipitation of solid. In order to slow down the reaction and observe its evolution, we examined the behavior of **3** by ^1H NMR spectroscopy at low temperatures. The addition of 1 drop of deuterated sodium hydroxide to a solution of **3** in CD_3OD maintained at −70 °C induces an immediate change in the spectrum: one of the two acetyl group signals initially present at 2.12 and 2.31 ppm disappears, leaving a single peak at 2.15 ppm while the two doublets due to C_3–H and C_2–H shift from 6.30 and 7.40 to 6.25 and 7.75 ppm, respectively. The spectrum observed is characteristic of **4**. The compound is stable under these conditions for a few hours. However, after some time at −30 °C, the spectrum exhibits a new pattern which corresponds to the

Scheme II



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(5) Enomoto, M.; Sato, K; Miller, E. C.; Miller, J. A. *Life Sci.* **1968**, *7*, 1025.
(6) Kawazoe, Y.; Araki, M.; Huang, G. F.; Okamoto, T.; Tada, M.; Tada, M. *Chem. Pharm. Bull.* **1975**, *23*, 3041
(7) Bailleul, B.; Galiegue, S.; Loucheux-Lefebvre, M. H. *Cancer Res.* **1981**, *41*, 4559.
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(9) Galiegue, S.; Bailleul, B.; Loucheux-Lefebvre, M. H. *Eur. J. Cancer* **1980**, *16*, 1283.
(10) Araki, M.; Kawazoe, Y.; Nagata, C. *Chem. Pharm. Bull.* **1969**, *17*, 1344.
(11) A preliminary report of the preparation of **5** has already appeared. See: Demeunynck, M.; Lhomme, M. F.; Lhomme, J. *Tetrahedron Lett.* **1981**, *22*, 3189.
(12) Bailleul, B.; Gailegue, S.; Loucheux-Lefebvre, M. H.; Demeunynck, M.; Lhomme, M. F.; Lhomme, *J. Chem. Biol. Int.*, in press.

dihydroxy derivative **2**, characterized by the two doublets at 6.50 and 7.90 ppm, attributed respectively to C_3–H and C_2–H. Above −10 °C, the spectrum becomes complex and precipitation is observed.

The nature of the precipitated solid was established by repeating the reaction on a larger scale at room temperature. HPLC and mass spectral analysis of the solid (30% yield based on **3**) indicate that it is a mixture of quinoline dimers belonging to the two following series: the azodiquinolines **7** and the pyridazino[3,4-*c*:5,6-*c*′]diquinolines **8**, present at different oxidation levels (Scheme II). The obtention of these ultimate products has been described previously, notably by Kosuge et al.,[13] as resulting from the basic treatment of **2**.

Thus these observations show that under basic conditions the hydrolysis of the diacetyl derivative is quite specific and rapid (instantaneous at −70 °C) and yields the monoacylated compound **4**. In a subsequent step the latter is deacetylated to the dihydroxy compound **2** (this process is rapid at −10 °C) which gives a number of secondary products including the dimers **7** and **8**. However, from a preparative point of view, this clearly shows that **4** cannot be obtained in basic medium, due to its extreme lability.

Hydrolysis under Acidic Conditions: CD_3OD–DCl. In acidic methanol (CD_3OD–DCl) **3** has enough stability to be observed by ^1H NMR spectroscopy at 30 °C, showing two characteristic acetate signals at 2.35 and 2.55 ppm (as discussed later these are shifted to higher fields as compared to the spectrum recorded at neutrality). Evolution is observed, and after 30 min **3** has totally disappeared, leaving signals corresponding to a mixture of the two monoacylated derivatives **4** and **5** (the two acetyl peaks of **3** at 2.35 and 2.55 ppm are replaced by a peak at 1.90 ppm corresponding to liberated acetic acid, and two new signals, respectively attributed to **4** and **5**, appear at 2.32 and 2.50 ppm). After a while the two new products slowly disappear, accompanied by the appearance of new signals. The mixture corresponding to a reaction time of 90 min at 25 °C is composed of the two hydroxyamino compounds **2** and **9** as indicated by the HPLC analysis (Scheme II). At no moment during the reaction can one observe the presence of a single monoacetyl compound.

However, as compared to the hydrolysis in neutral conditions the reaction is much less complex. It furnishes exclusively as primary products the two monoacetyl derivatives **4** and **5** at a rate which can be monitored at room temperature. We have already mentioned that the alkaline hydrolysis, although specific, is of no use from the preparative standpoint. This led us to make a more detailed investigation of the behavior of **3** under acidic conditions operating in a range of carefully controlled concentrations of acid.

Hydrolysis in Aqueous Hydrochloric Acid. Figure 1a–d represents the disappearance of the diacetyl compound **3** as a function of time in aqueous acid at various concentrations of hydrochloric acid. The study was carried at low concentrations of **3** (3×10^{-3} M). Also indicated are the percentages of the identified reaction products, as monitored by HPLC analysis.

Under very acidic conditions, i.e., in concentrated hydrochloric acid (12 N HCl), **3** rapidly disappears with a half-life of the order of 8 min. One single monoacetyl derivative, **5** appears on the way to the formation of the dihydroxy compound **2**.

When the acidity decreases (9 N HCl, Figure 1b) one observes the appearance of the second monoacetyl derivative **4**. In 6 N HCl, the two derivatives are formed in equal concentrations, and in 3 N HCl (Figure 1c) **4** is the major reaction product. Finally, in 1 N HCl (Figure 1d), **4** is the unique monoacetyl derivative formed. The half-life of **3** in these conditions is 6 min. Figure 2 summarizes the evolution of the concentrations of **4** and **5** in the whole range of concentrations studied. However, these curves cannot be interpreted directly in terms of selective deacylation leading either to **4** or **5**, depending upon HCl concentration. Both compounds may be further hydrolyzed in the medium to the dihydroxy compound **2** at rates which are different for the two species and which are pH dependent. In addition, the lability of all compounds under the HPLC analytical conditions renders a fully quantitative interpretation and rigourous kinetic treatment impossible. However, careful inspection of the data leads to a clear general qualitative conclusion. At low acid

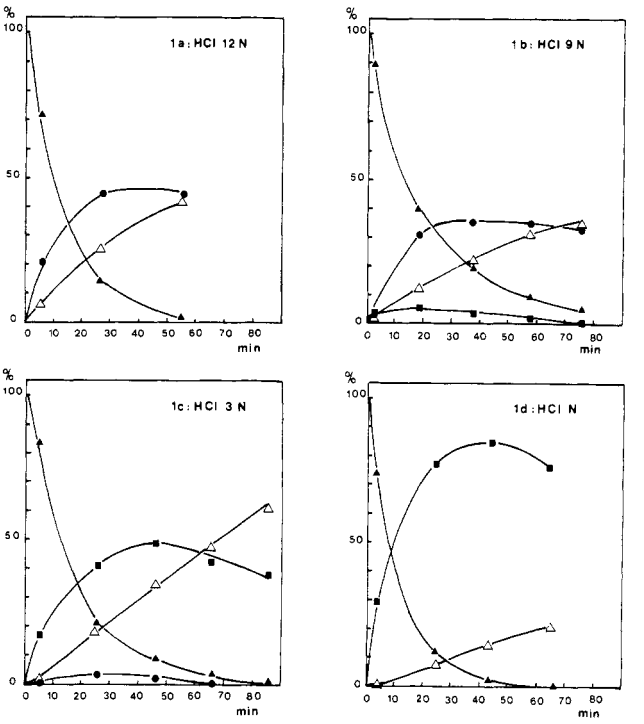


Figure 1. Solvolysis of **3** in acidic medium. Composition of the mixture as a function time: ▲, diacetyl **3**; ■, monoacetyl **4**, ●, monoacetyl **5**; △, dihydro **2**.

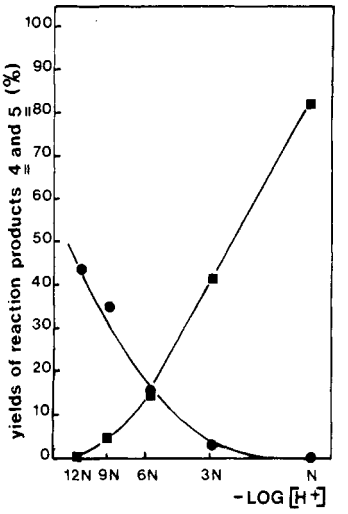


Figure 2. Selectivity in the hydrolysis of **3** under acidic conditions. Percentages of the monoacetyl derivatives **4** and **5** obtained at different HCl concentrations (■, monoacetyl **4**; ●, monoacetyl **5**).

(13) Kosuge, T.; Zenda, H.; Sawanishi, H. *Chem. Pharm. Bull.* **1969**, *17*, 2389 and preceding papers.

concentration (1 N HCl, Figure 1d) **4** is the unique product detected during the first minutes of the reaction, the dihydroxy compound **2** appearing slowly. After 10 min **4** is formed with an 85% yield. One can therefore confidently conclude that deacylation to **4** is selective under these conditions (we have checked that **5**, if formed, should be detected under the analytical conditions used). By contrast, in concentrated HCl (12 and 9 N HCl, Figures 1a,b) monoacetylated **5** is obtained with a yield which is always lower than 50%, and the formation of dihydroxy **2** is immediate. We have further checked that **5** is fairly stable under these conditions so that its rate of hydrolysis is such that it cannot account for the yields of **2** as observed.[14] This leaves the hypothesis that **2** is the result of immediate hydrolysis of the alternate monoacetyl derivative **4** formed by nonselective deacylation of **3**. This is further confirmed by the observation that **4**, when prepared in situ in a Me_2SO solution, instantaneously hydrolyses to **2** on addition of 12 N hydrochloric acid.

From all these observations one can conclude that the monohydrolysis of **3** is quite selective, giving **4** in dilute hydrochloric acid (1 N HCl). In concentrated HCl the reaction gives a mixture of the two monoacyl derivatives **4** and **5** in approximately a 50:50 ratio in all cases. **4** is immediately hydrolyzed, so that **5** is the single monoacylated derivate identified. These conclusion are quite in line with the result of the ^1H NMR study in acidic methanol.

The characteristic behavior of **3** in highly acidic media could be used to prepare **5** selectively. When diacetate **3** is treated with HCl in a nonnucleophilic solvent of low polarity such as $CHCl_3$, **5** is obtained as the hydrochloride which precipitates in the medium.[11]

Hydrolysis in a Weakly Acidic Medium. In less acidic medium, i.e., between pH 1 and 7, the half-life of the diacetyl compound **3** increases (19 min at pH 3 and 23 min at pH 5, at 20 °C). The reaction becomes more complex, and neither of the monoacetylated derivatives **4** and **5** can be observed, presumably due to their high reactivity. Thus, these reaction conditions are of no use from preparative point of view.

Conclusion

Diacetate **3**, which represents a model for the activated form of the carcinogen 4-NQO, is a very reactive compound which hydrolyzes to the monoacetates **4** and **5**. These compounds are themselves highly unstable. The primary hydrolysis step of **3** can be followed under acidic and basic conditions, while at neutrality and in a pH scale ranging from 7 to 1 the reaction is exceedingly complex. In the latter case the (hydroxyamino)acetate **6** could be isolated in the mixture. This compound results from an N–O cleavage reaction, for which no definite mechanistic conclusion can be drawn at the present time (for example, radical or ionic cleavage reaction[10,15]).

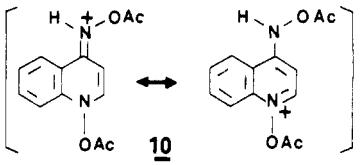
As far as the hydrolysis mechanism is concerned, we have shown that in basic, neutral, and weakly acidic media (from pH 12 to 1), the (hydroxyamino)acetate function at N-1 is selectively hydrolyzed. This means that this acetate group is more reactive toward bases or nucleophiles than the oxime acetate, i.e., that the carbonyl function is linked to a more electron-attracting substituent.[16] Indeed, the unusually high value of the hydroxyamino–carbonyl ester vibration in the IR at 1805 cm^{-1} probably reflects the strong electron-attracting effect of the quinoline moiety.[17] By comparison, the carbonyl of the oxime acetate appears at the more usual frequency of 1750 cm^{-1}.

Table I. Selected ^1H NMR Chemical Shifts (ppm) for Diacetyl Compound **3**[a]

	shift			
solvent	C_2H[b]	C_3H[b]	N_1 $OCOCH_3$	N_4 $OCOCH_3$
CD_3OD	7.40	6.30	2.31	2.12
CD_3OD, DCl[c]	9.00	7.15	2.55	2.35

[a] Spectrum recorded in neutral and acidic media. [b] Doublet $J_{2,3}$ = 8.0 Hz. [c] The solvent is about 0.3 M DCl in CD_3OD.

Under highly acidic conditions, however (below pH 1), the hydrolysis selectivity is no longer observed, and two monoacetates are formed. The most probable interpretation is that the species involved are no longer the same. It is now the protonated diacetate **10** which is hydrolyzed



10

without selectivity, the positive charge on the quinoline which constitutes part of the leaving group being delocalized on the N-1 and N-4 atoms. The ^1H NMR shift values measured for **3** in CD_3OD in the presence and in the absence of DCl are dramatically different, which agrees with this hypothesis (Table I).

Consequently, if a compound of the diacyl type **3** is brought into the presence of DNA, be it in vitro or in vivo, the reaction may well proceed directly with this species, but it is highly probable that some reaction could occur through the corresponding monoacyl derivatives of type **4** and **5**. Both **4** and **5** resemble the "activated" or "proximate" forms of various oncogens. Compound **4** possesses in one of its tautomers the *O*-hydroxyamino ester function present in the metabolite of aromatic amines[11] ($ArNH_2 \rightarrow Ar\text{–}N\text{–}O\text{–}COCH_3$).[18,19] Compound **5** is closely related to the presumed metabolite of oncogenic purine *N*-oxides.[20] We are presently studying the reactivity of these two monoacyl derivatives with nucleophiles and with the nucleotide bases.

Moreover, the two monoacetyl esters **4** and **5** are clearly quite unstable. This observation may have some significance in relation to the problem of the elucidation of the metabolic pathways for the conversion of 4-nitroquinoline 1-oxide (**1**) to the ultimate carcinogenic metabolite(s) which react(s) with cellular macromolecules. It has been demonstrated (Tada et al.)$_6$ that the (hydroxyamino)quinoline 1-oxide (**2**) is formed through enzymatic reduction of compound **1**. This intermediate is further transformed to a reactive ester(s), for which structures of types **3** and **4** have been postulated. From the present data, it seems quite unlikely that a diester, **3** could be formed metabolically from its highly unstable monoesters, **4** or **5**.[21]

(14) Demeunynck, M.; Lhomme, M. F.; Lhomme, J., to be submitted for publication.
(15) Comparable N–O cleavage leading to reduction products has been reported and fully discussed in the acyloxypurine series (see ref 20).
(16) Freeman, J. P. *J. Am. Chem. Soc.* **1958**, *80*, 5954.
(17) High-frequency absorption have also been reported for reactive *N*-oxide esters in the purine series. See, for example: Birdsall, N. J. M.; Lee, T. C.; Wolck, U. *Tetrahedron* **1971**, *27*, 5961.
(18) Miller, J. A. *Cancer Res.* **1970**, *30*, 559.
(19) Kriek, E.; Westra, J. G. In "Chemical Carcinogens and DNA"; Grover, P.L., Ed.; CRC Press: Cleveland, OH, 1979; Vol II, p1.
(20) Parham, J. C.; Templeton, M. A. *Tetrahedron* **1980**, *36*, 709 and references there in.

Table II. NMR Spectral Data

time, min	chemical shift, ppm		
	C_2H[a]	C_3H[a]	$COCH_3$
0	9.00	7.15	2.55, 2.35
25	9.00, 8.90, 8.70	7.15, 7.10	2.55, 2.50, 2.35, 2.32
30	8.90, 8.70	7.10[b]	2.50, 2.32
45	8.90	7.10	2.32
100	8.65	7.15, 7.05	

[a] C_2H and C_3H appear as doublets: $J_{2,3}$ = 8.0 Hz. [b] This signal at 7.10 integrates for two protons.

Experimental Section

^1H NMR spectra were recorded on a Perkin-Elmer 90-MHz spectrometer in the Fourier transform mode. Mass spectra were determined on Riber Mag 10-10 (electron impact) spectrometer.

Reversed-phase HPLC was performed with a μ-Bondapak RP 18 analytical column (Waters Associates) equipped with a Model 660 solvent programer and two M-6000 pumps (Waters Associates). The effluent was analyzed by a dual-wavelength detector (254 and 365 nm), and a linear gradient of solvents was used from 10% to 100% MeOH in water (pH 2.5, phosphoric acid) during 10 min with a flow rate of 2 mL/m.

Preparation of Starting Materials and Reference Compounds. 1-Acetoxy-4-(acetoxyimino)-1,4-dihydroquinoline (3) was prepared by the method described in the literature,[4,5] and its purity was checked by HPLC analysis.

1-Acetoxy-4-(hydroxyimino)-1,4-dihydroquinoline Hydrochloride (5). This compound was prepared as previously described.[11]

1-Hydroxy-4-(acetoxyimino)-1,4-dihydroquinoline (4). This derivative was required as a reference compound for NMR and HPLC analysis. It was obtained by the following methods.

(a) By Acetyl Transfer from Diacetyl Compound 3 to Thiophenol (NMR Studies). Compound 3 (20 mg, 0.07 mmol) was dissolved in $(CD_3)_2SO$ (0,6 mL) and then thiophenol (0.01 mL, 0.09 mmol) was added. After 5 min, the NMR spectrum indicated that it was a mixture of 4[2] and thioacetamide.

(b) By Acetyl Transfer from 3 to Piperidine. Piperidine (0.004 mL, 0.04 mmol) was added to a solution of 3 (10 mg, 0.03 mmol) in dimethyl sulfoxide (1 mL). After 5 min the solution was diluted to 10 mL with dimethylsulfoxide, and the mixture was analyzed by HPLC.

The chromatogram of monoacetyl derivative 4 is characterized by two major peaks which correspond to decomposition products instantly and repeatedly formed on the column.

Hydrolysis under Neutral Conditions. Compound 3 (10 mg, 0.03 mmol) was dissolved in 5 mL of methanol, and the solution was immediately diluted to 10 mL with a phosphate buffer (pH 7), so as to obtain a final concentration roughly equal to 3.5×10^{-3} M. The reaction was performed at room temperature, which remained near 20 °C.

The mixture was analyzed by HPLC at different time intervals. Pseudo-first-order rate constants are given in Figure 1 for disappearance of 3.

The presence of (hydroxyamino)quinoline acetate 6 was evidenced by HPLC analysis. The retention time and the ratio between the two signals corresponding to the absorptions at 254 and 365 nm were compared to those obtained for an authentic sample freshly prepared.[8]

Hydrolysis under Basic Conditions. (a) NMR Study. Diacetyl compound 3 (0.02 g, 0.07 mmol) was dissolved in CD_3OD (0.8 mL) under an inert atmosphere. The solution was subsequently frozen with liquid nitrogen, and 13 N NaOD (0.02 mL, 0.26 mmol) was added just before the NMR tube was inserted in the probe previously equilibrated at −70 °C. The temperature was then raised stepwise to 5 °C, recording the spectrum at each temperature.

Spectrum corresponding to 3, recorded before the addition of NaOD, at −70 °C: δ 8.30 (m, 1 H, C_5H), 7.50–7.10 (m, 4 H, Ar H and C_2H), 6.30 (d, 1 H, J = 8 Hz, C_3 H), 3.31 (s, 3 H, N_1–$OCOCH_3$), 2.12 (s, 3H, N–O–$COCH_3$).

After the addition of NaOD, −70 < T < −30 °C: δ 8.25 (m, 1 H), 8.05 (m, 1 H), 7.80–7.30 (m, 3H), 6.25 (d, 1 H, C_3 H), 2.15 (s, 3 H, $COCH_3$).

Evolution of the spectrum at −30 < T < −10 °C: δ 8.20–7.30 (m, 5 H, apparently 2 m at 8.20 and 8,10 and a d at 7.90), 6.50 (d, 1 H, J = 7.5 Hz, C_3H).

(b) Characterization of Reaction Products. A solution of 3 (30 mg, 0.11 mmol) in methanol was cooled to near 0 °C, and then 1 N sodium hydroxide (1 mL) was added. The solution was kept under vigourous stirring during 30 min, and then the red solid was filtered, washed twice with chloroform and dried (10 mg): MS, m/e (relative intensity) 330 (5), 316 (1), 314 (7), 300 (2), 298 (29), 284 (5), 282 (46), 254 (47), 242 (16), 226 (15), 144 (1), 141 (2), 128 (10), 127 (9), 114 (10), 102 (11). Peaks m/e 298, 314, and 330 correspond to pyridazino[3,4-c:5,6-c']diquinoline 8, mono-, di-, and trioxygenated. Peaks m/e 300 and 316 correspond to azodiquinoline 7, mono- and dioxygenated.

Hydrolysis under Acidic Conditions. (a) CD_3OD–DCl. Compound 3 (20 mg, 0.07 mmol) was dissolved in CD_3OD (0.8 mL). DCl (7 N, 0.04 mL, 0.28 mmol) was added under an inert atmosphere.

NMR spectra were measured at 30 °C at 5-min intervals. The main features of the spectra are given in Table II.

(b) Aqueous Hydrochloric Acid. For pH 2 and below, appropriate concentrations of HCl were used.[22] All reactions of diacetylated derivative 3 were performed with ~3.5×10^{-3} M solutions. The reactions were allowed to proceed at room temperature (18–20 °C). The mixtures were analyzed by HPLC. Products were identified by peak retention time and the ratio between the absorption intensities at the two different wavelengths ($R = OD_{254}/OD_{365}$).

Molar quantities were calculated from calibration standards: dihydroxylated compound 2, 1.4×10^{-3} M (retention time 7 min 10 s, R = 1.5); monoacetylated compound 5, 3.9×10^{-3} M (retention time 12 min 10 s, R = 1.6); compound 4, 3.7×10^{-3} M (retention 7 min 35 s and 8 min, R = 3.2 and 3.6, respectively); diacetylated compound 3, 3.8×10^{-3} M (retention time 10 min 50 s, R = 0.9).

All yields and recoveries are based on the initial weight of 3 used. All hydrolyses were carried out twice.

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(22) Buffered solutions with pH values between 2 and 7 were used.