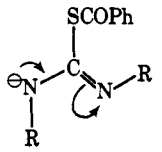
and in cases where these processes are slow as in **6** and **7**, then the isomerization can be rate determining in the overall S → N acyl transfer reaction.



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This study has explored in depth the reactions in aqueous solution of a series of simple *S*-benzoylisothioureas. Three modes of reaction have been observed, hydrolysis of the thiol ester function yielding benzoic acid and the parent thioureas, displacement of thiolbenzoate, and a facile intramolecular 1,3 S → N acyl transfer reaction yielding *N*-benzoylthioureas. The relative extent of these reactions occurring at any given pH is very strongly dependent on the structure of the compound involved. Even apparently very small structural changes such as addition of or changes in an *N*-alkyl substituent often lead to great changes in the rates, relative and absolute, of the reactions observed. The reasons for this great structural dependence have been examined in detail. The conclusions have generally been more semiquantitative than quantitative because of the complexity of the systems involved, appreciation of which emerged slowly as the work progressed. For instance, very little attempt has been made to compare rate constants for the various compounds because of the large number of unknown quantities involved in defining the geometric isomer distributions and isomer interconversion rates which appear critical to the interpretation of the reactions of the acyclic species. No attempt has been made to examine electronic effects either, *e.g.*, use of electron-withdrawing substituents, etc., although great variation in the types and rates of reactions would be expected from such changes. Prediction of such variation is certainly possible on the basis of the data from this study.

Acknowledgment. This work was supported from grants from the National Science Foundation and the National Institutes of Health.

Intramolecular General Base and Intermolecular Nucleophilic Catalysis of Carbonate Ester Hydrolysis. Hydrolysis of Ethyl 2-Hydroxy-5-nitrophenyl Carbonate

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Abstract: The rates of hydrolysis of ethyl 2-hydroxy-5-nitrophenyl carbonate have been determined in H_2O at 30°. Two pH-independent regions are present in the pH–rate constant profile. The rate constant for the pH-independent reaction at higher pH is 50 times greater than that for reaction at lower pH. The former reaction is most likely a phenoxide ion catalyzed attack of H_2O at the ester carbonyl rather than a hydroxide ion catalyzed hydrolysis of the un-ionized ester. With azide and imidazole the rate constant for reaction with the ionized ester is considerably less than that for reaction with the un-ionized species, but the rate constants in the latter case are nearly the same as with ethyl 2-methoxy-5-nitrophenyl carbonate and ethyl 3-nitrophenyl carbonate. Thus a neighboring phenoxide ion greatly retards reaction with these nucleophiles. Hydroxide ion catalysis is also greatly reduced. However, morpholine catalysis displays little sensitivity to the nature of the leaving group in this series. Solvent isotope effects ($k_B^{H_2O}/k_B^{D_2O}$) close to unity were found for morpholine and pyrrolidine catalysis of the hydrolysis of the ionized species, and for imidazole-catalyzed hydrolysis of both ionized and un-ionized species. Nucleophilic catalysis is thereby indicated in all cases. *N*-Methylimidazole is a good catalyst for hydrolysis of the un-ionized compound, but catalysis of the hydrolysis of the ionized ester could not be detected. A likely possibility in hydrolysis of the ionized species is expulsion of the 4-nitrocatechol monoanion *via* a tetrahedral intermediate.

The presence of a neighboring hydroxyl group markedly accelerates the alkaline hydrolysis or methanolysis of aliphatic esters.[2,3] Esters possessing a neighboring phenolic hydroxyl group also hydrolyze with enhanced rates.[4-9] The most likely mechanism of this reaction has been considered to be a phenoxide ion general base catalyzed attack of water at the carbonyl of the ester.[4,5]

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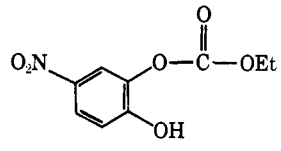
Nitrophenyl carbonate esters are quite sensitive to reaction with water.[10] A large pH-independent region is observed in the pH–rate constant profiles for hydrolysis of these compounds. As a consequence, it was thought that interesting effects of a neighboring phenolic hydroxyl group might be observed in the hydrolysis of a nitrophenyl carbonate ester. We have therefore studied the reactions of ethyl 2-hydroxy-5-nitrophenyl carbonate (I).



I

Experimental Section

Materials. *o*-(4-Nitrophenylene) carbonate was prepared by a previous procedure,[10,11] mp 100–100.5°; lit.[10] mp 99–100°.

Ethyl 2-hydroxy-5-nitrophenyl carbonate (I) was prepared by refluxing *o*-(4-nitrophenylene) carbonate for 10 min in ethanol which had previously been dried by distillation over sodium. The excess solvent was removed at 30–40° at reduced pressure. The yellow oil quickly solidified and was dissolved in a little anhydrous diethyl ether. Dry hexane was added until the mixture clouded; when the preparation was cooled, the white product crystallized. Recrystallization by the same method and subsequent drying at reduced pressure at 56° yielded material which melted at 89–90°. *Anal.* Calcd for $C_9H_9NO_6$: C, 47.58; H, 3.99; N, 6.17. Found: C, 47.74; H, 4.25; N, 6.17.

Ethyl 3-nitrophenyl carbonate (II) was prepared by the addition of *m*-nitrophenol (Aldrich, 3.48 g, 0.025 mol) to ethyl chloroformate (2.71 g, 0.025 mol) and pyridine (1.98 g, 0.025 mol) in anhydrous ether (30 ml). The mixture was stirred for 5 hr. The pyridine salt was removed by filtration, and petroleum ether was added until precipitation occurred. After a brief period at 0°, the solid residue was filtered off and recrystallized from anhydrous ether–petroleum ether (2:1). The pale yellow solid was dried at reduced pressure at room temperature, mp 50.5–51°; lit.[12] 52.5–53°. An infrared spectrum showed no phenolic OH.

Ethyl 2-methoxy-5-nitrophenyl carbonate (III) was first prepared by refluxing ethyl 2-hydroxy-5-nitrophenyl carbonate (0.10 g, 4.4×10^{-4} mol) with dimethyl sulfate (0.025 ml, 2.3×10^{-4} mol) and anhydrous potassium carbonate (0.066 g, 4.8×10^{-4} mol) in a small volume of acetone which had been dried by standing over anhydrous K_2CO_3. The dimethyl sulfate was added by microsyringe, and the reaction mixture was protected from atmospheric moisture with a $CaCl_2$ drying tube. The inorganic residue was removed by filtration and washed with dry acetone. After removal of some of the solvent by distillation, dry petroleum ether was added until precipitation started. A small amount of orange material deposited at this stage. The mixture was filtered, and further petroleum ether was added to the almost colorless solution. Refrigeration resulted in the formation of white crystals which melted at 99–100°. Infrared analysis showed little phenol remained. A subsequent preparation of the same compound used 5-nitroguaiacol (*vide infra*) as starting material. This was treated with ethyl chloroformate and pyridine in the manner described for ethyl *m*-nitrophenyl carbonate. The compound obtained by ether–hexane recrystallization had an infrared spectrum and melting point (100–100.5°) identical with that obtained for material prepared by the first described procedure (mmp 99–100°). *Anal.* Calcd for $C_{10}H_{11}NO_6$: C, 49.80; H, 4.60; N, 5.81. Found: C, 49.77; H, 4.79; N, 5.87.

5-Nitroguaiacol was prepared from guaiacol (K & K Laboratories) by nitration of the acetate and subsequent hydrolysis,[13] mp 104–105° (recrystallized from benzene); lit.[13] 104.5°.

4-Nitroguaiacol was prepared from 4-nitroveratrole (Aldrich, recrystallized material) by a reported procedure,[14] mp 100.5–102°; lit.[14] 101–102°.

Buffer Solutions. Standard HCl and KOH solutions were made from "Dilut-it" concentrated analytical reagents (J. T. Baker) by dilution with boiled deionized water. Standard KH_2PO_4, H_3BO_3, sodium formate, and KCl solutions were prepared from the corresponding A. R. grade materials. Imidazole (Eastman Organic Chemicals) was recrystallized from benzene. Morpholine, pyridine (Merck, A. R.), and pyrrolidine (Aldrich) were distilled before use and standard solutions prepared. Buffer solutions were prepared stoichiometrically by mixing appropriate volumes of the standard solutions and were made up with KCl to give ionic strengths of 0.5. Deuterium oxide solutions were prepared in similar fashion using 99.9% D_2O from Bio-Rad Laboratories. Deuterium chloride (38% in D_2O, Bio-Rad) was diluted and standardized against KOH.

Spectral Properties. The ultraviolet spectra of ethyl 2-hydroxy-5-nitrophenyl carbonate in both the ionized and un-ionized forms were taken at wavelengths above 300 nm using a Zeiss PMQ II spectrophotometer. The cell compartment was thermostatted at 30 ± 0.05°, and measurements were made immediately after injection of 25 μl of a solution of the compound in acetonitrile into 3 ml of buffer: $\lambda_{max}^{pH\ 3.66}$ 312 nm, log ε 3.92; $\lambda_{max}^{pH\ 7.62}$ 395 nm, log ε 4.24. The buffers used were 0.1 *M* acetate and 0.1 *M* phosphate, respectively, with ionic strengths adjusted to 0.5 with KCl.

Similarly, the ultraviolet spectrum of ethyl 2-methoxy-5-nitrophenyl carbonate at pH 7.62 showed a single maximum in the region above 250 nm; λ_{max} 310 nm, log ε 3.13. For the hydrolysis product, 5-nitroguaiacol, at 30° in 0.05 *M* KOH the following data were obtained: $\lambda_{max}^{0.05\ M\ KOH}$ nm (log ε): 262 (4.13), 320 (3.75), 416 (3.63); compared with reported values[15] $\lambda_{max}^{0.1\ N\ NaOH}$ nm (log ε): 228 (3.98), 263 (4.06), 418 (3.54).

The ultraviolet spectrum obtained from 4-nitroguaiacol prepared in this work was quite distinct from that of 5-nitroguaiacol, $\lambda_{max}^{0.01\ M\ NaOH}$ nm (log ε): 266 (3.81), 435 (4.32); reported[15] $\lambda_{max}^{0.1\ N\ NaOH}$ nm (log ε): 266 (3.73), 435 (4.26).

The ultraviolet spectrum of ethyl 3-nitrophenyl carbonate in the range 230–400 nm has a single λ_{max} at about 260 nm (log ε 3.96).

Kinetic Measurements. Hydrolysis rates of the various carbonate esters were measured by following either the absorbance decrease due to loss of substrate or the rise in absorbance due to phenol or phenolate products. Both processes were found to obey pseudo-first-order kinetics. A Zeiss PMQ II and a Gilford 2000 recording spectrophotometer were both used for this purpose. For measuring the hydrolysis of ethyl 2-hydroxy-5-nitrophenyl carbonate it was preferable to follow the decline in absorbance at 392 nm, corresponding to loss of ester, because of a slow decline in absorbance at 430 nm for solutions containing 4-nitrocatechol at pH >8. At low pH, the rise in absorbance at 345 nm due to production of 4-nitrocatechol gave a greater absorbance change than loss of substrate at 312 nm. Rates were measured, however, at all four possible λ_{max} values. In borate buffers it was found that 4-nitrocatechol monoanion exhibited a λ_{max} at 390 instead of 430 nm, possibly because of complex formation, so that production of phenolate in this medium could not be followed. The hydrolysis rates of ethyl 2-methoxy-5-nitrophenyl carbonate were measured by observing production of either the phenolate at 414 nm or the phenol at 380 nm. Ethyl 3-nitrophenyl carbonate hydrolysis rates were determined by following either formation of phenol (333 nm) or phenolate (392 nm).[16] Substrate was generally introduced as 10–30 μl of solution in dry acetonitrile into 3 ml of buffer using a Hamilton syringe. Quartz 1-cm cells with Teflon stoppers were employed. Cuvettes were thermostatted at 30 ± 0.05° by circulating water from a Haake Model F circulating bath through a constant temperature cell holder. The pH of each solution was measured at the end of the reaction using a Radiometer 22 pH meter. Rate constant measurements were invariably made in duplicate or triplicate, and values of k_{obsd}, the pseudo-first-order rate constants, were calculated with an IBM 360-40 computer, using a rigorous least-squares procedure.

pK_a Determination. A value of 5.48 at 30° and μ = 0.5 *M* was determined for the pK_a of ethyl 2-hydroxy-5-nitrophenyl carbonate by measuring the absorbance at 400 and 312 nm of solutions con-

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Table I. Second-Order Rate Constants (M^{-1} sec^{-1}) for Catalysis of the Hydrolysis of Ethyl Nitrophenyl Carbonate Esters at 30°

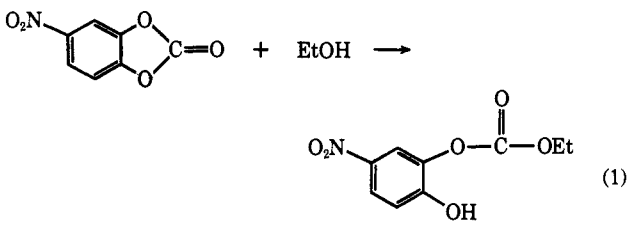
Base	pK_a	I (OH)	I (O^-)	II (H)	III (OMe)
H_2O		2.88×10^{-8} [a]	1.44×10^{-6} [a]		
OH^-			8.0×10^{-2}	3.1	1.62
N_3^- [b]		2.7×10^{-3}	*c*	2.8×10^{-3}	
Pyridine	5.30[d]	4.7×10^{-4}	*e*		
Imidazole	7.05[d]	5.64×10^{-2}	2.0×10^{-4}	8.2×10^{-2}	7.2×10^{-2}
N-Methylimidazole	7.10[d]	4.4×10^{-2}			
Morpholine	8.60[d]	*f*	5.0×10^{-1}	8.1×10^{-2}	1.36×10^{-1}
Pyrrolidine	11.05[d]		2.17	15	14
2,5-Dimethyl-pyrrolidine	11.10[d]		1.43×10^{-1}		

[a] $k_0/55.5$. [b] Azide catalysis was investigated in phosphate buffers (0.1 *M*, pH 6.62) and borate buffers (0.1 *M*, pH 8.97). Ionic strength = 0.5 *M*. [c] No evidence for catalysis in borate buffers (see *b*). An upper limit on the rate constant can be calculated to be 5×10^{-5} M^{-1} sec^{-1}. [d] pH 30° of half-neutralized buffers. [e] All data may be explained in terms of pyridine reacting with the unionized form of I. An upper limit on the rate constant can be calculated to be 4×10^{-5} M^{-1} sec^{-1}. [f] All data may be explained in terms of morpholine reacting with the ionized form of I. An upper limit on the rate constant can be calculated to be 7×10^{-1} M^{-1} sec^{-1}.

taining identical amounts of material in ten different 0.1 *M* acetate and phosphate buffers (pH range 3.66–7.62). The compound was introduced as 25 μl of solution in acetonitrile, and measurements were made as rapidly as possible after addition to minimize loss due to hydrolysis.

Results

The identity of the compound obtained by ethanolysis of *o*-(4-nitrophenylene) carbonate was conclusively established to be ethyl 2-hydroxy-5-nitrophenyl carbonate by methylation of the phenolic hydroxyl group



(1)

and comparison with the compound obtained from the known 5-nitroguaiacol by treatment with ethyl chloroformate. The two compounds were identical. It was further established that alkaline hydrolysis of the compound prepared in the former manner yielded 5-nitroguaiacol, whose infrared and ultraviolet spectrum (particularly the latter) were quite distinct from those obtained for 4-nitroguaiacol. The structures of the two isomeric nitroguaiacols have been well established previously by classical methods.[13,14]

In Figure 1, a plot is shown of k_{obsd} for hydrolysis of ethyl 2-hydroxy-5-nitrophenyl carbonate at 30° *vs.* pH. The values of k_{obsd} were obtained by extrapolation to zero buffer concentration. The line is theoretical and was constructed from eq 2 and the rate constants in

$$k_{obsd} = k_0\left(\frac{a_H}{K_a + a_H}\right) + k_0'\left(\frac{K_a}{K_a + a_H}\right) + k_{OH}(OH^-) \quad (2)$$

Table I. In eq 2, k_0 and k_0' are the rate constants for spontaneous hydrolysis of the un-ionized and ionized species respectively, k_{OH} is the second-order rate constant for hydroxide ion catalysis of ionized species hydrolysis, and K_a is the dissociation constant of the phenolic hydroxyl group. This pH–rate constant profile has two pH-independent regions. The pH-independent reaction at higher pH proceeds 50 times more rapidly than does the reaction at lower pH. The D_2O solvent isotope effect measured in a half-neutralized 0.02 *M* H_3BO_3 buffer is $k_0^{H_2O'}/k_0^{D_2O'} = 2.3$ for the ionized species.

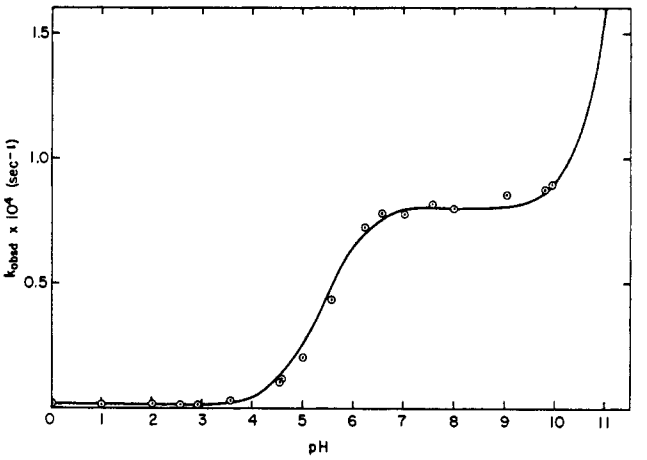


Figure 1. Plot of k_{obsd} for hydrolysis of ethyl 2-hydroxy-5-nitrophenyl carbonate at 30° (μ = 0.5) *vs.* pH. Rate constants were obtained by extrapolation to zero buffer concentration.

Various nucleophiles react readily with ethyl 2-hydroxy-5-nitrophenyl carbonate. In Figure 2 is shown a plot of k_{obsd} *vs.* total imidazole concentration. It can be seen that facile catalysis takes place. The slopes of plots of k_{obsd} *vs.* imidazole free base concentration increase as the pH decreases, indicating that imidazole catalysis is greater with the un-ionized ester. This assumes that the base form of imidazole catalyzes the hydrolysis of both species. A general acid catalyzed hydrolysis of the anion, kinetically equivalent to a general base catalyzed hydrolysis of the un-ionized ester is not likely in view of the lack of acid catalysis in the hydrolysis of these compounds.[10] At pH 2, catalysis by imidazolium ion was not observed, indicating that ImH^+ is not catalytically active toward the un-ionized ester. Imidazole catalysis is described by eq 3 or a

$$k_{obsd} = k_0'' + k_{Im}'(Im)\left[\frac{a_H}{K_a + a_H}\right] + k_{Im}''(Im)\left[\frac{K_a}{K_a + a_H}\right] \quad (3)$$

kinetically equivalent equation, where (Im) is the con-

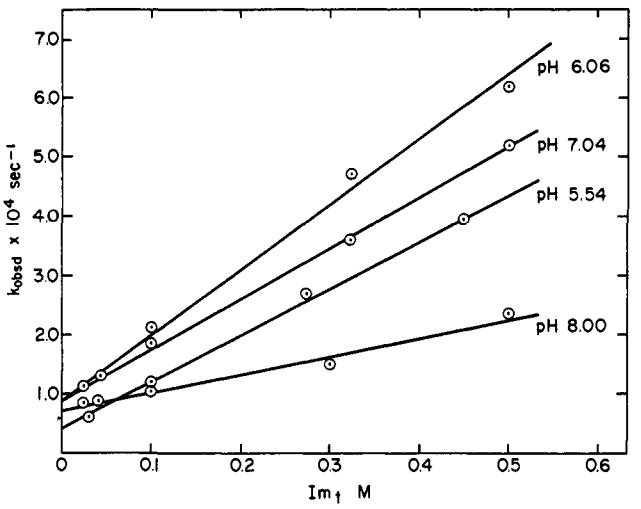


Figure 2. Plot of k_{obsd} for hydrolysis of ethyl 2-hydroxy-5-nitrophenyl carbonate at 30° (μ = 0.5 maintained constant with KCl) *vs.* total imidazole concentration (Im + ImH$^+$). The data at pH 5.54 were obtained in acetate buffer.

centration of the free base form of imidazole and K_a is the dissociation constant of the phenolic hydroxyl group of the ester. Plots of k_{obsd} *vs.* (Im) give k_0'' as the intercept and k_{Im} (total) as the slope. Then plotting k_{Im} (total) *vs.* ($K_a/(K_a + a_H)$) as in Figure 3 gives k_{Im}' as the intercept where ($K_a/(K_a + a_H)$) is zero and k_{Im}'' as the intercept where ($K_a/(K_a + a_H)$) is unity. The value of k_{Im}'' determined in this manner is in good agreement with that found with imidazole in borate buffers at pH 9–10. Similar treatment of the data obtained in D_2O gave, within error, second-order rate constants for both species identical with those determined in H_2O.

Catalysis by pyrrolidine and 2,5-dimethylpyrrolidine likewise gives D_2O solvent isotope effects of unity, indicating that nucleophilic catalysis is occurring. Morpholine catalysis is only slightly reduced in D_2O ($k_B^{H_2O}/k_B^{D_2O}$ = 1.2).

Rate constants for a series of nucleophiles are presented in Table I. Identical second-order rate constants were calculated from plots of k_{obsd} *vs.* free base concentration for both imidazole and morpholine catalysis of the hydrolysis of II and III when the pH was varied. It will be noted that *N*-methylimidazole has a catalytic rate constant for hydrolysis of the un-ionized species of I comparable to that for imidazole, but in contrast with imidazole, catalysis could not be detected in reactions of the ionized ester. *N*-Methylimidazole buffers were employed at pH 6.01, 7.10, and 8.10. Also, the reaction was studied in 0.02 *M* half-neutralized borate buffer (pH 8.95) where imidazole catalysis is easily detected. All data may be explained in terms of *N*-methylimidazole reacting with the unionized species. It can be calculated that if reaction of the ionized species is being catalyzed, the catalytic rate constant must be less than 3×10^{-5} M^{-1} sec^{-1}. 2,5-Dimethylpyrrolidine has a second-order rate constant 14 times less than that of pyrrolidine. Catalysis by collidine buffers was not observed. The presence of steric hindrance further supports a mechanism involving nucleophilic catalysis. Formate and acetate had no effect on the rate constants in the buffer concentration range 0–0.5 *M*.

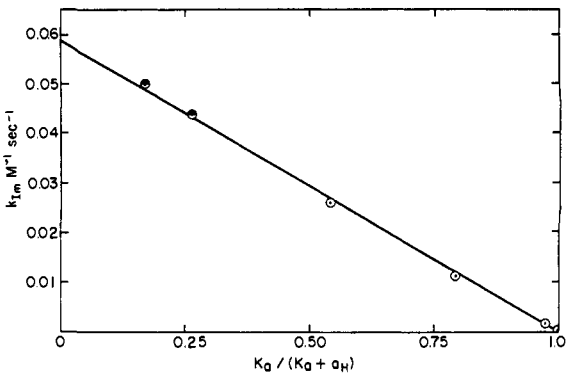
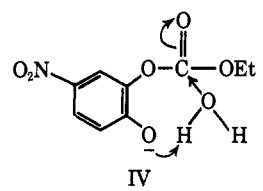


Figure 3. Plot of k_{Im} (total) for imidazole-catalyzed hydrolysis of ethyl 2-hydroxy-5-nitrophenyl carbonate at 30° *vs.* $K_a/(K_a + a_H)$ where K_a is the dissociation constant of the phenolic OH group. The points ⊖ were obtained at pH values outside the buffering range of imidazole, but pH remained reasonably constant during the kinetic measurements.

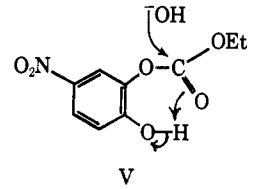
Discussion

A plateau region in the pH–rate constant profile has been found in the hydrolysis of both bis(4-nitrophenyl) carbonate and *o*-(4-nitrophenylene) carbonate.[10] These pH-independent reactions undoubtedly involve attack of water at the ester carbonyl. This would appear to be a generally favorable mechanism in the hydrolysis of nitrophenyl carbonate esters. In the case of ethyl 2-hydroxy-5-nitrophenyl carbonate there are two plateau regions in the profile (Figure 1). As with the other nitrophenyl carbonate esters, water attack at the carbonyl must be occurring, but it will be noted that the rate constant k_0' for the pH-independent reaction taking place at higher pH is 50 times greater than that for the reaction occurring at low pH. A very similar pH–rate constant profile was reported for hydrolysis of *p*-nitrophenyl 5-nitrosalicylate, but the difference in magnitude of the two pH-independent rate constants was less (30 times).[4]

The much greater rate constant at pH values above the pK_a of the neighboring hydroxyl group might indicate that attack of water is being catalyzed by the phenoxide ion. However, two kinetically equivalent possibilities exist. Mechanism IV involves general base



IV



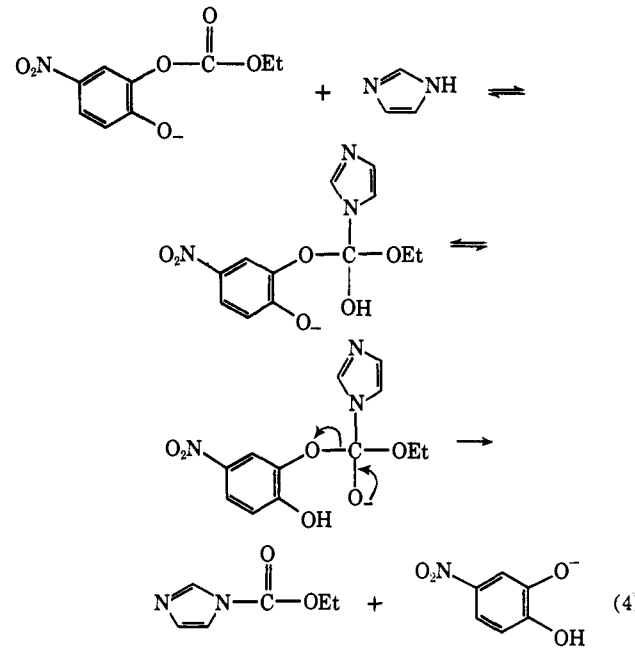
V

catalysis by the ionized species and in mechanism V general acid, specific base catalysis takes place. Both of these mechanisms could give rise to the much slower reaction in D_2O than in H_2O ($k_0^{H_2O'}/k_0^{D_2O'}$ = 2.3). However, the evidence points strongly to IV as the correct mechanism. Table I compares second-order rate constants for catalysis by various nucleophiles of the hydrolysis of the ionized and unionized species of I, ethyl *m*-nitrophenyl carbonate, and ethyl 2-methoxy-5-nitrophenyl carbonate. In the latter two compounds the neighboring hydroxyl group has been replaced by hydrogen and methoxy, respectively. It will be noted that while the pH-independent reaction is considerably

faster with the ionized ester, this enhanced reactivity is not seen in reaction with nucleophiles. Azide reacts at least 50 times more rapidly with the un-ionized species and has similar reactivity toward the unsubstituted compound (II). A neighboring phenoxide ion could not facilitate attack of this nucleophile, but intramolecular general acid catalysis by the phenolic group would be expected to enhance the rate. Since this does not occur in reactions of nucleophiles, it is unlikely that the pH-independent spontaneous reaction involves that type of catalysis. Thus, the most likely mechanism is IV in which the neighboring phenoxide ion catalyzes attack of H_2O as a general base. A similar mechanism was preferred by Bender, *et al.*,[4] in the hydrolysis of *p*-nitrophenyl 5-nitrosalicylate on the basis of similar evidence and reasoning. Capon and Ghosh[5] also prefer an intramolecular general base mechanism in the hydrolysis of phenyl salicylate and catechol monobenzoate.

In view of the D_2O solvent isotope effect of unity, imidazole catalysis is most likely proceeding by a nucleophilic mechanism. The reaction should be considerably slower in D_2O if proton transfer occurs in the critical transition state. Imidazole shows a marked preference for reaction with the un-ionized ester. The large rate difference of 280 times is due to a retarding effect of the phenoxide ion rather than enhancement by the un-ionized phenolic hydroxyl since replacement of the OH group by either H or OCH_3 gives similar rate constants for imidazole catalysis. The difference in rate constants for imidazole catalysis of the hydrolysis of the two species was much less in hydrolysis of *p*-nitrophenyl 5-nitrosalicylate (6 times).[4] A large retarding effect of the phenoxide ion on the attack of negatively charged nucleophiles can also be seen in the hydrolysis of I with azide and hydroxide ion. This is undoubtedly due in part to electrostatic repulsion of the negatively charged nucleophile, but the large rate retardation in the case of the neutral nucleophile imidazole indicates that other factors are also important.

It might be expected that the ability of nucleophiles to catalyze the hydrolysis of the anionic species would be reduced in comparison with the unionized species since the leaving group is of much higher pK_a, the nitrocatechol dianion having a pK_a of approximately 11.[17] It is therefore of considerable interest that significant nucleophilic catalysis by amines as weakly basic as imidazole (pK_a = 7.05) takes place. Thus, it is apparent that carbonate diesters of nitrocatechol are quite susceptible to nucleophilic attack by nitrogen bases even when the pK_a of the leaving group greatly exceeds that of the catalyzing base. With carboxylic esters it would appear that in bimolecular reactions when the pK_a of the leaving group exceeds that of the catalyzing base by 2–3 pK_a units imidazole catalysis will not be strictly nucleophilic but will proceed through other mechanistic pathways involving proton transfer.[18] In the hydrolysis of esters of nitrocatechol monoanion a favorable pathway is available in which the anion is protonated to afford a much better leaving group. Partitioning of a tetrahedral intermediate would then actually involve expulsion of nitrocatechol monoanion as in eq 4. The tetrahedral intermediate could then readily break down



(4)

to products rather than reverting to starting material. A kinetic equivalent reaction involving the un-ionized ester and imidazole anion is, of course, also a possibility. However, it was established that the neutral base form of imidazole is the catalyst in hydrolysis of II and III. Support for these mechanistic possibilities is provided by the fact that *N*-methylimidazole, which does not have a proton that can dissociate, is a good catalyst for hydrolysis of the un-ionized species but does not detectably catalyze hydrolysis of the ionized species.

It will be noted that the considerably more basic amine morpholine exhibits only small difference in catalytic ability towards the ionized species of I, the unsubstituted compound II, and the methoxy derivative III, indicating that in these cases the rate is not very sensitive to the nature of the leaving group. This would be the case if the leaving groups are electronically similar or if attack of amine at the carbonyl is rate limiting with little bond breaking in the critical transition state. There is only a difference of 5.4 in the catalytic rate constants for morpholine and pyrrolidine, bases differing in pK_a by 2.5 pK_a units, which might indicate that the N–C bond is not well developed in the transition state. However, the reactivity of the more weakly basic imidazole and pyridine is much less.

As in the case of imidazole catalysis, the D_2O solvent isotope effect for morpholine catalysis is reasonably close to unity ($k_B^{H_2O}/k_B^{D_2O}$ = 1.2). Thus proton transfer is probably not taking place as part of the rate-limiting step. If the phenoxide ion of I is being protonated to afford a better leaving group it must occur in a discrete step after attack by amine at the carbonyl. As a consequence, an intermediate would be required in the reaction. Johnson[19] has pointed out that direct displacement reactions might be most likely for carbonate or carbamate ester hydrolysis because of the possibility of back bonding of the lone-pair electrons of the alkoxyl oxygens into the relatively low energy π^* orbital of the carbonyl group.[20] Thus, while the amine catalysis data can be reasonably explained in terms of a tetrahedral

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(18) T. C. Bruice and S. J. Benkovic, *J. Amer. Chem. Soc.*, **86**, 418 (1964); J. F. Kirsch and W. P. Jencks, *ibid.*, **86**, 833 (1964).

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intermediate, a direct displacement reaction might also be possible with the relative amounts of bond breaking and bond formation with the nucleophile varying as the pK_a of the attacking amine varies. However, in the case of imidazole catalysis this would require direct expulsion of a much more basic species. Also, it would then be expected that *N*-methylimidazole would catalyze hydrolysis of the ionized species as well as imidazole which is not the case. Therefore, it is most likely that a tetrahedral intermediate is being formed.

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"Abbreviated" Dinucleosides of Thymidine and Deoxyuridine and Their Photoproducts[1]

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Abstract: The photodimerization of two thymines and of thymine and uracil held in close proximity by a ribofuranose backbone has been examined. "Abbreviated" dinucleosides 5′-deoxy-5′-(1-thyminyl)thymidine (**3a**) and 2′,5′-dideoxy-5′-(1-thyminyl)uridine (**3b**) have been synthesized *via* ring closure of the appropriate 5′-*N*-(β-methoxy-α-methylacryloyl)ureidodeoxynucleosides **2a** and **2b**. Intermediates **2a** and **2b** were prepared by the treatment of 5′-amino-5′-deoxythymidine (**1a**) and 5′-amino-2′,5′-dideoxyuridine (**1b**), respectively, with β-methoxy-α-methylacryloyl isocyanate. Direct irradiation of **3a** at 300 nm in dilute aqueous solution leads exclusively to an internal cis-syn photodimer (*cis*-**4a**, where the additional cis refers to the relation of the furanose oxygen to the 2- and 2′′-carbonyls). Acetone-sensitized photolysis of **3b** leads to an internal cis-syn photodimer (*cis*-**4b**) and an internal trans-syn photodimer (**7** with the furanose oxygen in cis relation to the 2-carbonyl and in trans relation to the 2′′-carbonyl) in approximately a 1:1 ratio. The results are informative with regard to base stacking and favored conformations.

The isolation of an internal cis-syn dimer from direct photolysis of 1,1′-trimethylenebisthymine (Thy-C_3-Thy) at 300 nm in dilute aqueous solution[2a] prompted us to devise a model system more closely related to DNA[3] and to examine its photochemistry. In "abbreviated" dinucleosides,[4] ribonucleosides, or deoxyribonucleosides containing an extra base on the 5′-carbon, the two bases have the possibility of existing in a stacked conformation approximately 3.4 Å apart, which is in the range generally observed for the interplanar distances between bases in nucleic acids.[5] We now describe the syntheses of "abbreviated" dinucleosides 5′-deoxy-5′-(1-thyminyl)thymidine (**3a**) and 2′,5′-dideoxy-5′-(1-thyminyl)uridine (**3b**) and their photochemistry. Ureidodeoxynucleosides **2a** and **2b** were prepared by the reaction of β-methoxy-α-methylacryloyl isocyanate with 5′-amino-5′-deoxythymidine (**1a**) and 5′-amino-2′,5′-dideoxyuridine (**1b**), respectively. Ring closure of intermediates **2a** and **2b** in the presence of ammonium hydroxide[6,7] gave the corresponding "abbreviated" dinucleosides **3a** and **3b**.

When compound **3a** was irradiated at 300 nm in dilute aqueous solution (1.1×10^{-3} *M*) while sparging with deoxygenated nitrogen, the ultraviolet absorption at 262 nm decreased to 7% of its original value after 20 hr. Thin layer chromatography on cellulose indicated only one product in addition to a small amount of recovered **3a**. Fractional crystallization from water served to separate **3a** (2%) from internal photodimer **4a** (88%). The structure of **4a** was established by chemical and spectroscopic means.

The anti-type dimers can be ruled out due to the geometrical restraint caused by the attachment of both thymine rings to the single carbohydrate moiety. A single-crystal X-ray analysis was not possible due to dissociation of **4a** under the influence of X-rays.[8] The

(1) (a) For part VIII, the preceding paper in the series on Synthetic Spectroscopic Models Related to Coenzymes and Base Pairs, see J. A. Secrist III and N. J. Leonard, *J. Amer. Chem. Soc.*, **94**, 1702 (1972); (b) the present paper may be regarded as part IX in this series.

(2) (a) N. J. Leonard, K. Golankiewicz, R. S. McCredie, S. M. Johnson, and I. C. Paul, *J. Amer. Chem. Soc.*, **91**, 5855 (1969); (b) internal photodimerization of 5-EtUra-C_3-Ura and Thy-C_3-Ura has also been reported: K. Golankiewicz and L. Strekowski, *Bull. Acad. Polon. Sci., Ser. Sci. Chim.*, **19**, 97 (1971).

(3) For reviews of the products of ultraviolet radiation of nucleic acids and their components, see: E. Fahr, *Angew. Chem., Int. Ed. Engl.*, **8**, 578 (1969); J. G. Burr, *Advan. Photochem.*, **6**, 193 (1968).

(4) "Doubled-headed" deoxyribonucleosides have been made in the Merck Sharp and Dohme Research Laboratories bearing adenine and/or thymine moieties at the 1′ and 5′ positions: R. Fecher, K. H. Boswell, J. J. Wittick, and T. Y. Shen, *J. Amer. Chem. Soc.*, **92**, 1400 (1970); *Carbohyd. Res.*, **13**, 105 (1970). The authors are grateful to Dr. Shen for providing a preprint of their communication while our work was in progress. For a review, see T. Y. Shen, *Angew. Chem., Int. Ed. Engl.*, **9**, 678 (1970). The present authors prefer the designation "abbreviated" in referring to coenzyme or dinucleoside models of this type. In keeping with the symbolism of pyrimidine photoproducts suggested by Dr. Waldo Cohn, Director of the Office of Biochemical Nomenclature, National Research Council, the following shortened forms may be applied: for **3a**, Thy(1dRib5)Thy; **3b**, Ura(1dRib5)Thy; *cis*- and *trans*-**4a**, Thy[1dRib5]Thy(*c*) (the brackets indicate cyclobutane dimer formation and (*c*) indicates its cis geometry; the relationship of the furanose oxygen to the 2- and 2′′-carbonyls is not specified in this terminology); *cis*- and *trans*-**4b**, Ura[1Rib5]Thy(*c*); **6** and **7**, Ura[1d-Rib5]Thy(*t*).

(5) M. Sundaralingam, *Biopolymers*, **7**, 821 (1969).

(6) G. Shaw and R. N. Warrener, *J. Chem. Soc.*, 157 (1958).

(7) T. Ukita, A. Hamada, and M. Yoshida, *Chem. Pharm. Bull.*, **12**, 454 (1964).

(8) We are grateful to Professor I. C. Paul, University of Illinois, for carrying out and monitoring the X-irradiation. Crystals of **4a** which had been exposed to X-irradiation for 1 week had dissociated to an