RBAChE, which when treated with 2-PAM or TMB-4, recovered only an additional 18% of the total activity. These data suggest that irreversible, postinhibitory mechanisms are operative immediately following inhibition by **2a+**. Again, the oxime-mediated reactivation kinetics of **2a±** and **2a−** inhibited RBAChE were similar. Mole fraction calculations were conducted on the oxime-mediated process to give $k_{oxime} = 3.78 \times 10^{-2}$ min^{-1}, which compares reasonably with the observed (4.57×10^{-2} min^{-1}), although these values are not in as precise agreement as the k_0 values.

The percent spontaneous reactivation following inhibition of RBAChE by **2a+** was 12%, leaving 88% in the inhibited or non-reactivated form (Table II). This aspect is further highlighted by the fact that k_{NR} is approximately 9-fold k_0, indicating that RBAChE phosphorylated by **2a+** undergoes very little spontaneous reactivation in the first 30 min. In contrast, k_0 and k_{NR} values obtained from **2a−** inhibited RBAChE were similar, suggesting that spontaneous reactivation and non-reactivation were competing postinhibitory processes. The larger mole fraction of **2a−** inhibited RBAChE compared to **2a±** inhibited RBAChE contributed to making k_{NR} analogous to the k_0 and k_{oxime} values for racemic-inhibited RBAChE. The theoretical k_{NR} based on mole fraction for **2a±** inhibited RBAChE is 1.57×10^{-2} min^{-1}, comparable with the observed 1.37×10^{-2}.

Our chemical model studies on the mechanism of non-reactivatability showed that an $-OCH_3$ moiety was released in the reaction of 2-PAM with *O,O,S*-trimethyl phosphorothiolate. No 2-PAM methyl ether was observed, suggesting that the mechanism favored hydrolysis via first formation of the putative phosphorylated 2-PAM, over a dealkylation pathway. This result is consistent with hydrolysis data for methamidophos (*O,S*-dimethyl phosphoramidothiolate), which undergoes preferential P–OMe scission in aqueous base.[27] Therefore, a majority of the non-reactivation probably proceeds via hydrolysis of the O-methyl group rather than the S-methyl, which does not necessarily follow prediction based upon phosphorylation leaving group ability.[25]

Conclusions

The kinetic data indicate that phosphorothiolates bearing a center of asymmetry at phosphorus differ in their postinhibitory mechanisms and their inhibitory potency. Despite the greater sensitivity of RBAChE toward inactivation by **2a−**, the RBAChE could recover from poisoning by this stereoisomer. Conversely, RBAChE phosphorylated by **2a+** may be more susceptible to a cumulative inactivation, owing to inability of the enzyme to be reactivated.

Acknowledgment. This work was supported by the National Institute of Environmental Health Sciences (Grant ES04434), and this support is gratefully acknowledged. The authors thank Debra Quinn for technical assistance and Loyola University of Chicago for the purchase of the Varian NMR spectrometer used in this study and partial support through the BRSG.

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Intramolecular Ureido and Amide Group Participation in Reactions of Carbonate Diesters

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Abstract: The cyclization of ethyl and phenyl 2-ureidophenylcarbonates in H_2O at 30 °C involves two discrete steps with benzoxazolinone as the final product. The formation of benzoxazolinone is quantitative. Phenol is released in the initial step from both esters in an apparent OH^--catalyzed reaction, which shows that intramolecular nucleophilic attack by the ureido group is via an anionic species. The pH–rate constant profile for the second step in the reaction of the ethyl ester is sigmoidal with $pK_{app} = 8.9$. The initial reaction involves a rearrangement, and the neighboring phenoxide ion of the intermediate participates in the second step. In view of the D_2O solvent isotope effect ($k_{H_2O}/k_{D_2O} = 1.2$), this participation must be via a nucleophilic mechanism. The second step of the reaction of the phenyl ester, in which benzoxazolinone is formed, involves an apparent OH^--catalyzed reaction of a cyclic intermediate. This intermediate was identified as *N*-carbamoylbenzoxazolinone, which thereby indicates that the initial nucleophilic attack is by nitrogen through a 5-membered-ring transition state. *p*-Nitrophenol release from *p*-nitrophenyl 2-ureidophenylcarbonate is only 18-fold faster than phenol release from the corresponding phenyl ester. Ratios of k_{OH}(ortho) for phenol release from the 2-ureido-substituted esters to k_{OH}(para) for OH^--catalyzed hydrolysis of the corresponding 4-ureido-substituted esters are approximately 10^4 in all cases. In the intramolecular nucleophilic reactions of the ureido-substituted carbonate esters, a neutral species reaction is not observed, even at pH values as low as 3, in contrast with substituted benzoate esters having phenolic leaving groups. Also, in the apparent OH^--catalyzed reactions of the carbonate diesters, only one cyclic product is obtained, whereas both oxygen and nitrogen attack occur in the nucleophilic reactions of carboxylate esters. The neighboring amide group of *p*-nitrophenyl *o*-(carboxamido)phenylcarbonate participates with nitrogen attack and apparent OH^- catalysis. Intramolecular attack of nitrogen provides a rate enhancement of 10^3 in the release of *p*-nitrophenol over the OH^--catalyzed hydrolysis of the para-substituted compound. Thus, in the intramolecular nucleophilic reactions of the carbonate diesters, nitrogen anion attack takes place preferentially when such attack is sterically favored (five-membered-ring transition state) and when there is an equal opportunity for oxygen or nitrogen attack.

A number of important enzymes that catalyze carboxylation reactions require biotin as a cofactor.[1,2] All of these processes occur by two partial reactions in which biotin serves as a HCO_3^- acceptor and carboxyl carrier. The reactions require adenosine 5′-triphosphate (ATP) and involve a carboxybiotin intermediate (eq 1). *N*-Carboxybiotin was isolated from reactions catalyzed by β-methyl crotonyl-COA carboxylase[2] and will serve as a substrate in enzymatic reactions.[3] However, it was pointed out

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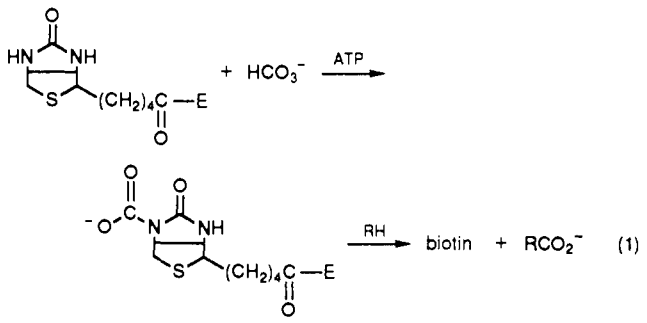
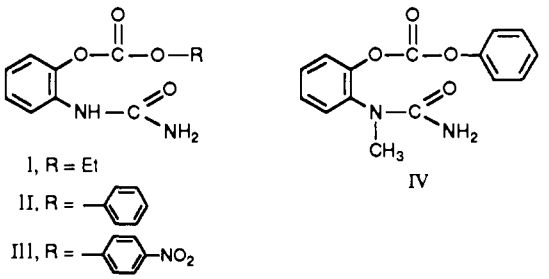


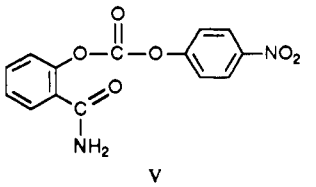
(1)

by Bruice and Hegarty[4] that the reaction could proceed with initial carboxylation of the ureido oxygen followed by rearrangement to give the *N*-carboxy derivative. The requirement for ATP may indicate that CO_2 is activated by formation of a carbonyl phosphate intermediate which then undergoes nucleophilic attack by the ureido function of biotin.[5]

Before the enzyme reactions can be understood, the mechanisms by which a ureido group functions as a nucleophile toward activated acyl groups must be established and the magnitudes of the rate constants for such reactions determined. Chemical intramolecular reactions bear a close analogy to the intracomplex reactions of enzymes[6] and can be employed as models to gain insight into the chemistry of enzymatic reactions. A neighboring ureido group is an excellent nucleophile in reactions of carboxylate esters having various leaving groups.[7] The oxygen adjoining the carbonyl group of carbonic acid derivatives should lead to significant differences in the transition-state structure for carbonate diesters as compared with carboxylate esters. Some striking mechanistic differences have been found in the intramolecular aminolysis reactions of the two types of compounds.[8,9] Therefore, we have investigated the intramolecular nucleophilic attack by a neighboring ureido group in reactions of the carbonate diesters I–IV, and for comparison purposes, the rate constants for hydrolysis of the corresponding *p*-ureido derivatives have been determined.



Nucleophilic attack of nitrogen might be reasonably expected with I–III since a kinetically favored five-membered-ring transition state can be formed, in contrast with the 7-membered-ring transition state required for oxygen attack. Consequently, we have also studied the amide-substituted carbonate diester V, with which there is an equal steric opportunity for nitrogen and oxygen attack.



V

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Experimental Section

Materials. *o*-Ureidophenol. Sublimed *o*-aminophenol (10 g, 0.092 mol) from Aldrich was dissolved in 700 mL of 1:1 glacial acetic acid–water. A solution of potassium cyanate (0.2 mol) in water was then added dropwise. The solution was stirred for 3 h and was then extracted 3 times with diethyl ether. The ether layer was rotary evaporated, leaving an orange oil. A 10% sodium bicarbonate solution was then added to the thick oil, and the precipitate was collected. The material was recrystallized once from methanol and then several times from acetone–hexane to yield white needles which melted at 158–160 °C. Anal. Calcd for $C_7H_8N_2O_2$: C, 55.26; H, 5.26; N, 18.42. Found: C, 55.28; H, 5.41; N, 18.34. The infrared spectrum (KBr) had strong absorbance at 1650 cm^{-1} (C=O).

***p*-Ureidophenol.** The method used in the synthesis of *o*-ureidophenol was also followed in the preparation of *p*-ureidophenol using sublimed *p*-aminophenol (Aldrich). The compound melted at 168–170 °C. The infrared spectrum (KBr) had strong absorbance at 1660 cm^{-1} (C=O).

Ethyl 2-Ureidophenylcarbonate (I). *o*-Ureidophenol (0.012 mol) and distilled triethylamine (0.013 mol) were dissolved in 30 mL of dry tetrahydrofuran (THF). The solution was cooled at 4 °C in an ice bath. Distilled ethyl chloroformate (0.013 mol) in 20 mL of dry THF was added dropwise over a period of 20 min. The reaction mixture was stirred for 12 h at 4 °C. The solution was then filtered, and the filtrate was evaporated to dryness. The resultant solid was triturated with dry diethyl ether and was recrystallized twice from chloroform and once from acetone–hexane to yield white needles which melted at 134–136 °C: ^1H NMR δ 8.2 (1 H), 1.25 (3 H); IR (KBr) ν 1790, 1660 (C=O), 1240 cm^{-1} (C—O). Anal. Calcd for $C_{10}H_{12}N_2O_4$: C, 53.57; H, 5.40; N, 12.50. Found: C, 53.62; H, 5.36; N, 12.23.

Ethyl 4-ureidophenylcarbonate was prepared by a method identical with that used in the synthesis of ethyl 2-ureidophenylcarbonate except that the final solid was not triturated with ether but was recrystallized twice from acetone–hexane. The white needles melted at 156–158 °C: ^1H NMR δ 8.7 (1 H), 1.25 (3 H); IR (KBr) ν 1750, 1680 (C=O), 1275 cm^{-1} (C—O). Anal. Calcd for $C_{10}H_{12}N_2O_4$: C, 53.57; H, 5.40; N, 12.50. Found: C, 53.52; H, 5.49; N, 12.66.

Phenyl 2-ureidophenylcarbonate (II) was prepared by reaction of *o*-ureidophenol with phenyl chloroformate (Eastman) employing the same procedure used for the synthesis of I. *o*-Ureidophenol (0.012 mol) and distilled triethylamine (MCB) (0.013 mol) were dissolved in 30 mL of dry tetrahydrofuran. The solution was then cooled at 4 °C in an ice bath. Distilled phenyl chloroformate (0.013 mol) in 20 mL of dry THF was added dropwise over a period of 20 min. The reaction mixture was then stirred for 12 h at 4 °C. The solution was filtered, and the filtrate was evaporated to dryness. The solid residue was recrystallized from acetone–hexane. After removal of a cyclized compound by fractional crystallization, the ester was recrystallized twice more from an acetone–hexane mixture. The white needles melted at 142–144 °C: ^1H NMR δ 8.8 (1 H); IR (KBr) ν 1775, 1680 (C=O), 1245 cm^{-1} (C—O). Anal. Calcd for $C_{14}H_{12}N_2O_4$: C, 61.76; H, 4.41; N, 10.29. Found: C, 61.85; H, 4.58; N, 10.38.

Cyclic Derivative of II. The cyclized compound isolated in the synthesis of II and separated from II by fractional crystallization from an acetone–hexane mixture was recrystallized 5 times from acetone–hexane. The white needles melted at 146–149 °C: IR (KBr) ν 1795, 1730 cm^{-1} (C=O). Anal. Calcd for $C_8H_6N_2O_3$: C, 53.93; H, 3.37; N, 15.73. Found: C, 53.85; H, 3.49; N, 15.65.

Phenyl 4-ureidophenylcarbonate was prepared by reacting 4-ureidophenol with phenyl chloroformate by the method employed in the synthesis of I. The white needles melted at 187–190 °C after recrystallization from acetone–hexane: ^1H NMR δ 8.8 (1 H). Anal. Calcd for $C_{14}H_{12}N_2O_4$: C, 61.76; H, 4.41; N, 10.29. Found: C, 61.51; H, 4.36; N, 10.27.

***p*-Nitrophenyl 2-Ureidophenylcarbonate (III).** *o*-Ureidophenol (0.012 mol) was dissolved in 35 mL of THF. Sublimed *p*-nitrophenyl chloroformate was dissolved in 20 mL of THF and was added to the *o*-ureidophenol dropwise over a period of 20 min. The mixture was then refluxed for 3 h and was allowed to stand at room temperature for 16 h. The mixture was rotary evaporated to give a semisolid which was dried under vacuum for 24 h. The material was washed and triturated with diethyl ether to remove any contaminating *p*-nitrophenol or *p*-nitrophenyl chloroformate. Other contaminants were then removed by sublimation at 65 °C for 16 h. The carbonate ester, which remained unsublimed, melted at 134–138 °C: IR (KBr) ν 1750, 1700, 1200 cm^{-1}. Anal. Calcd for $C_{14}H_{11}N_3O_6$: C, 53.00; H, 3.49; N, 13.25. Found: C, 52.88; H, 4.01; N, 12.93. Inclusion of an equivalent of triethylamine in the reaction mixture led to the formation of a cyclic derivative which was identical with that formed in the preparation of II.

***p*-Nitrophenyl 4-ureidophenylcarbonate** was prepared from *p*-nitrophenyl chloroformate and 4-ureidophenol by the same method used in

the synthesis of phenyl and ethyl 4-ureidophenylcarbonates. However, the solid obtained by rotary evaporation after filtration was triturated with diethyl ether. Subsequently, the solid was recrystallized from acetone. The resultant crystals melted at 232–234 °C (dec): ^{1}H NMR δ 8.4 (1 H). Anal. Calcd for $C_{14}H_{11}N_3O_6$: C, 53.00; H, 3.44; N, 13.25. Found: C, 52.83; H, 3.55; N, 13.42.

Phenyl 2-(*N*-Methylureido)phenylcarbonate (IV). *o*-Aminophenol (55 g) was refluxed with 31.4 mL of iodomethane in 1300 mL of ethanol for 5 h. The ethanol was removed by rotary evaporation. Aqueous 5% sodium carbonate (300 mL) was added to the oil residue. This solution was extracted 3 times with 200 mL of ether. The ether extract was dried over magnesium sulfate. The ether was then rotary evaporated. The brown solid residue was dissolved in boiling hexane and decolorized with charcoal. After filtration, crystallization occurred when the solution was allowed to stand. The crystalline material melted at 85–90 °C. The *o*-(*N*-methylamino)phenol (7.5 g) was dissolved in 250 mL of a 1:1 acetic acid–water mixture. Potassium cyanate (15 g) in 30 mL of water was added dropwise over 30 min, and the solution was then stirred for 3 h. The solution was then extracted with 250 mL of ether. The ether layer was evaporated, and aqueous 10% $NaHCO_3$ solution (300 mL) was added to the residue. This solution was extracted with ether. The ether was rotary evaporated, and the residue was recrystallized from an acetone–hexane mixture. The 2-(*N*-methylureido)phenol melted sharply at 135 °C. 2-(*N*-Methylureido)phenol (0.83 g, 0.005 mol) and triethylamine (0.005 mol) were dissolved in 50 mL of THF. An equimolar quantity of phenyl chloroformate (0.78 g) in 50 mL of THF was added dropwise with stirring. The mixture was stirred overnight at room temperature. The mixture was then filtered, and the THF removed by rotary evaporation. The oil residue could not be crystallized and decomposed upon attempted distillation. The material was purified by repeatedly dissolving it in cyclohexane and adding hexane to give back the oil. Anal. Calcd for $C_{15}H_{14}N_2O_4$: C, 62.93; H, 4.90. Found: C, 62.54; H, 5.17.

***p*-Nitrophenyl 2-(Carboxamido)phenylcarbonate (V).** Salicylamide (2.74 g, 0.02 mol) and pyridine (1.58 g, 0.02 mol) were dissolved in 100 mL of dry ether. *p*-Nitrophenyl chloroformate (4.02 g) in ether was added dropwise with stirring over a period of 2 h. The mixture was then stirred for an additional 2 h. The solution was decanted from the precipitated pyridine hydrochloride and then rapidly filtered. The ether filtrate was rotary evaporated. The residue was recrystallized several times from an ethyl acetate–hexane mixture and melted at 99 °C: ^{13}C NMR δ 172.6, 161.1 (C=O). Anal. Calcd for $C_{14}H_{10}N_2O_6$: C, 55.63; H, 3.34; N, 9.27. Found: C, 55.49; H, 3.39; N, 9.15.

***p*-Nitrophenyl 4-(carboxamido)phenylcarbonate** was prepared by the same method as V employing 4-hydroxybenzamide. After recrystallization from an ethyl acetate–hexane mixture, the compound melted at 148–150 °C: ^{13}C NMR δ 168.5, 164.4 (C=O). Anal. Calcd for $C_{14}H_{10}N_2O_6$: C, 55.63; H, 3.34; N, 9.27. Found: C, 55.66; H, 3.26; N, 9.17.

***N*-Carbamoylbenzoxazolinone.** Equivalent amounts of benzoxazolinone and triethylamine were dissolved in ether. An equivalent of freshly sublimed *p*-nitrophenyl chloroformate in ether was then added dropwise over a period of 15 min. The mixture was stirred for an additional 2 h and filtered. The ether was then removed by rotary evaporation. The material was dissolved in chloroform and extracted with an aqueous 0.01 M sodium carbonate solution. The chloroform layer was dried with sodium sulfate, filtered, and then rotary evaporated. After recrystallization from an ether–hexane mixture, the product melted at 127–129 °C. The carbamate ester (1 g) was dissolved in chloroform. This solution was added slowly to 3.0 g of gaseous ammonia dissolved in 50 mL of chloroform. The mixture was allowed to stir for 20 min and was filtered. The chloroform was removed by rotary evaporation. The residue was recrystallized from a chloroform–hexane mixture and melted at 147–150 °C. *N*-Carbamoylbenzoxazolinone has a reported mp of 144–146 °C.[10] The same compound was also prepared from the corresponding ethyl ester (mp 80 °C) by treatment with gaseous ammonia in chloroform.

NMR Measurements. ^{1}H and ^{13}C NMR spectra were obtained with a Brucker-250 spectrometer. DMSO was employed as the solvent. In all cases the spectra were consistent with the assigned structure. Chemical shifts are reported in reference to TMS.

HPLC Measurements. HPLC determinations were conducted on an Altex Model 310 chromatographic system, using an Ultrasphere ODs 15- × 0.46-cm analytical column and a 1:1 methanol/water eluant. In the assay procedure, 1×10^{-5} mol of each sample was added to 1 mL of 1 M KOH and incubated for 10 min at room temperature (22 °C). Subsequently, 1 mL of 1 M HCl was added to the basic solution followed by 2 mL of HPLC-grade methanol. The samples were then injected and run at a pressure of 2100 psi with a flow rate of 1 mL/min. Absorbance was monitored at 280 nm.

Kinetic Measurements. The rates of reaction of the ortho-substituted compounds I–IV were measured with a Beckman Model 25 recording spectrophotometer or a Model D110 Durrum–Gibson stopped-flow spectrophotometer, by following the appearance of their respective intermediates and the benzoxazolinone product at 272 or 280 nm or, in the case of III, *p*-nitrophenol at 400 or 320 nm. The rates of hydrolysis of the para-substituted compounds were measured by following the appearance of *p*-ureidophenol at 290 nm or *p*-nitrophenol at 400 or 320 nm. The reactions of V and the corresponding *p*-carboxamide derivative were monitored by following the appearance of *p*-nitrophenol at 400 or 330 nm at 30 °C, μ = 0.5 M. All buffer solutions were maintained at a constant ionic strength of 1.0 or 0.5 M with KCl. A typical kinetic run employing the Beckman recording spectrophotometer was initiated by injecting 20–30 μL of a 2×10^{-2} M ester stock solution in acetonitrile (0.005 M with III) into 2 mL of buffer maintained at 30 °C.

Reactions that were too rapid to be monitored with a conventional spectrophotometer were followed using a Durrum–Gibson stopped-flow spectrophotometer. The desired concentration of substrate was dissolved in an HCl solution in which it is reasonably stable. This solution was introduced into one of two identical drive syringes. The other syringe contained the appropriate buffer. The drive syringes were suspended in a water trough whose temperature was maintained at 30 °C. Absorbance changes after mixing were recorded on a Hewlett-Packard storage oscilloscope (Model 1207B). Reaction mixture pH values were measured with a Radiometer Type PHM 22r pH meter at 30 °C. The values of p*D* were calculated by employing the glass electrode correction equation of Fife and Bruice.[11] In calculating second-order rate constants for OH^- catalysis, k_{OH}, the ion product of water (K_w) was 1.47×10^{-14}.

Product Isolation. Ethyl 2-ureidophenylcarbonate (0.67 g) was dissolved in 7 mL of ethanol. This solution was then added with stirring to 100 mL of water. The pH of the solution was maintained at pH 10 by the addition of 1 M KOH. The reaction was allowed to proceed to completion (1 h). Enough 1 M HCl was then added to bring the pH to 7.0. The aqueous solution was extracted twice with chloroform, and the chloroform layer was rotary evaporated. The solid residue was recrystallized from water. The crystals melted at 137–139 °C. The melting point, IR, HPLC, and UV spectra were identical with those of an authentic sample of benzoxazolinone. A mixture melting point revealed no depression.

The final product from the cyclization of V was also isolated. The ester (1.0 g) was dissolved in 10 mL of acetonitrile. To this was added 500 mL of water. The solution was maintained at 70 °C for ~8 h. The solution was concentrated to about 10 mL and allowed to cool. The white crystalline product was removed by filtration. The material was washed thoroughly with water and then dried. The compound melted at 141 °C, identical with salicylamide. A mixture melting point with an authentic sample of salicylamide revealed no depression. The product also has an absorbance spectrum identical with that of salicylamide (λ_{max} is 325 nm in 0.1 M KOH). Salicylic acid melts at 160 °C.

The ester V was also dissolved in a minimum amount of acetonitrile and mixed with 5 mL of 0.001 M KOH solution. After 5 min the solution was extracted several times with ether. The ether extracts were washed with cold water and dried over anhydrous sodium sulfate. The ether was removed by flash evaporation. The residue was recrystallized from ether. The material melted sharply at 222 °C, which is identical with the melting point of an authentic sample of 1,3-benzoxazine-2,4-dione.[12] A mixture melting point determination showed no depression. The infrared spectrum of the isolated material was also identical with that of the synthetically prepared 1,3-benzoxazine-2,4-dione.

Results

The cyclization of I proceeds in H_2O at 30 °C in two distinct steps. The plot of log k_{obsd} vs pH for the first step in Figure 1 is linear with a slope of 1.0, $k_{OH} = 1.35 \times 10^4$ M^{-1} s^{-1}. The value of k_{OH} for hydrolysis of ethyl 4-ureidophenylcarbonate is 0.89 M^{-1} s^{-1}. Experimental points in Figure 1 were obtained from measurements either in KOH solutions or by extrapolation to zero buffer concentration. There is a change in the slope from 1.0 to 0 in the plot of log k_{obsd} vs pH for the second step in the reaction of the ethyl ester (Figure 1). The value of pK_{app} is 8.9, and the limiting rate constant $k_o = 3.70 \times 10^{-3}$ s^{-1}. The pH-independent

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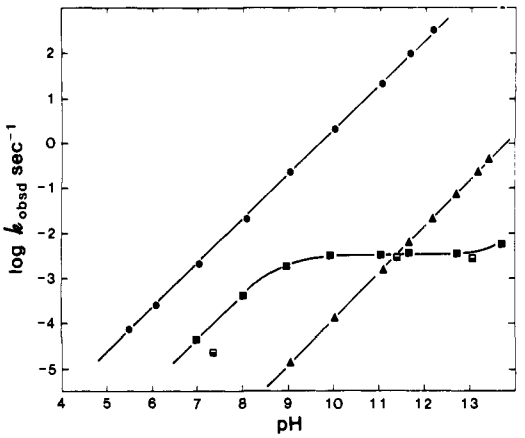


Figure 1. Plots of log k_{obsd} vs pH for the first (●) and second (■) steps in the reaction of ethyl 2-ureidophenylcarbonate (I) in H_2O and in D_2O (▣) at 30 °C, μ = 1.0 M with KCl. The plot of log k_{obsd} vs pH is also given for hydrolysis of ethyl 4-ureidophenylcarbonate (▲) at 30 °C.

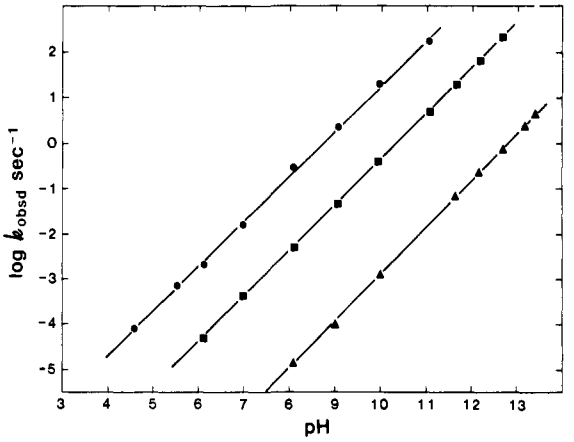


Figure 2. Plots of log k_{obsd} vs pH for the first (●) and second (■) steps in the reaction of phenyl 2-ureidophenylcarbonate (II) in H_2O at 30 °C, μ = 1.0 M with KCl. The plot of log k_{obsd} vs pH is also given for hydrolysis of phenyl 4-ureidophenylcarbonate (▲) at 30 °C.

reaction at high pH proceeds at nearly the same rate in D_2O as in H_2O (k_{H_2O}/k_{D_2O} = 1.2). The UV spectrum of the product of the first step showed the presence of a phenolic-OH group. However, the spectrum of the product of the second step was not that of ureidophenol but rather was identical with that of benzoxazolinone. That benzoxazolinone is indeed the final product was shown conclusively by isolation and comparison with an authentic sample.

In Figure 2 is shown a plot of log k_{obsd} vs pH for phenol release from II in H_2O at 30 °C. The plot is linear with a slope of 1.0, which indicates apparent OH^- catalysis. The second-order rate constant, k_{OH}, has the value 1.26×10^5 M^{-1} s^{-1}. In comparison, k_{OH} for OH^--catalyzed hydrolysis of phenyl 4-ureidophenylcarbonate is 8.3 M^{-1} s^{-1}. Again, all data in Figure 2 were obtained from measurements in KOH solutions or by extrapolation to zero buffer concentration. A slower second observed step in the reaction of II also occurs with OH^- catalysis as shown in Figure 2, k_{OH} = 2.92×10^3 M^{-1} s^{-1}. This value is nearly identical with that of the cyclic derivative (k_{OH} = 2.73×10^3 M^{-1} s^{-1}) prepared from *o*-ureidophenol and phenyl chloroformate (see Experimental Section). The extinction coefficient at 270 nm of the intermediate produced in the reaction of II is also identical with that of the compound obtained synthetically from II. Therefore, the ring closure is a quantitative reaction. Final product analysis in the reaction of II was conducted by HPLC analysis. The results, shown in Figure 3, identify the products of the reaction as phenol and benzoxazolinone. Thus, the reaction of II proceeds in two stages with initial formation of a cyclic derivative which then reacts to give a stable final product (benzoxazolinone) which is identical with that formed from I. Synthetically prepared *N*-carbamoylbenzoxazolinone had spectral characteristics identical with the intermediate produced in the reaction of II and hydrolyzed to benzoxazolinone with the same k_{OH} value at 30 °C (3.1×10^3 M^{-1} s^{-1}). The analogous *N*-methylated derivative (IV) prepared from *o*-(*N*-methylamino)phenol releases phenol with k_{OH} = 67.6 M^{-1} s^{-1}. Thus, the intramolecular nucleophilic attack in the reaction of II is by the *N*-phenyl nitrogen.

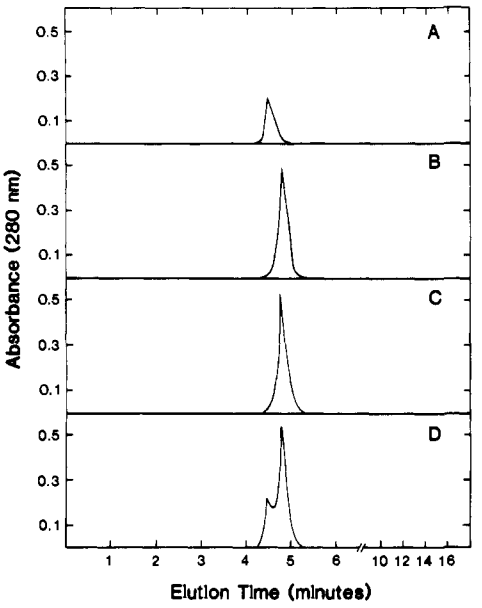


Figure 3. HPLC spectra of the reaction products from compounds I and II and the standards: A, phenol; B, benzoxazolinone (Aldrich sample); C, the isolated reaction product from compound I; D, the reaction products from compound II. The concentrations of I and II and the standards A and B were identical. The conditions and procedure are given in the Experimental Section.

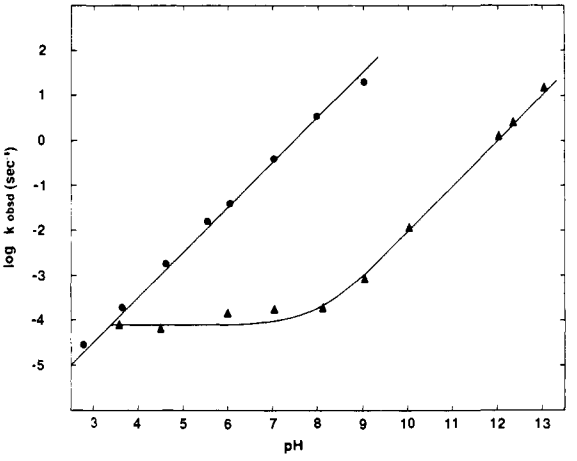


Figure 4. Plots of log k_{obsd} vs pH for release of *p*-nitrophenol from *p*-nitrophenyl 2-ureidophenylcarbonate (III) (●) and *p*-nitrophenyl 4-ureidophenylcarbonate (▲) in H_2O at 30 °C, μ = 1.0 M with KCl.

The plot of log k_{obsd} vs pH for release of *p*-nitrophenol from III in Figure 4 is linear with a slope of 1.0 in the pH region 3–9, k_{OH} = 2.3×10^6 M^{-1} s^{-1}. The value of k_{OH} for OH^--catalyzed hydrolysis of *p*-nitrophenyl 4-ureidophenylcarbonate is 64 M^{-1} s^{-1}. A pH-independent water reaction was also observed in the hydrolysis of *p*-nitrophenyl 4-ureidophenylcarbonate at pH < 8 with a rate constant (k_o) of 7.7×10^{-5} s^{-1}. Rate constants for the ureido-substituted esters are summarized in Table I.

The initial reactions of the 2-ureido-substituted esters (I–III), in which apparent OH^- catalysis is observed, can be considered to proceed via the ionized species, so that eq 2 is followed, where

$$k_{obsd} = k_2 K_a (OH^-)/K_w \quad (2)$$

K_a is the dissociation constant of the ureido group and K_w is the ion product of water. The second-order rate constants (k_{OH}) for

Table I. Rate Constants for Reactions of *o*- and *p*-Ureido-Substituted Carbonate Diesters in H_2O at 30 °C (μ = 1.0 M with KCl)

ureido	ester	k_{OH}, M^{-1} s^{-1}	k_0, s^{-1}	pK_{app}
ortho	ethyl (1st rxn)	1.35×10^4		
	ethyl (2nd rxn)	2.95×10^2	3.70×10^{-3}	8.9
para	ethyl	8.90×10^{-1}		
ortho	phenyl (1st rxn)	1.26×10^5		
	phenyl (2nd rxn)	2.92×10^3		
para	phenyl	8.28		
ortho	nitrophenyl	2.29×10^6		
para	nitrophenyl	6.4×10^1	7.7×10^{-5}	

Table II. Second-Order Rate Constants for General Base-Catalyzed Reactions of Carbonate Diesters of *p*-Ureidophenol in H_2O at 30 °C (μ = 1.0 M with KCl)

ester	base	$10^2 k_B$, M^{-1} s^{-1}
ethyl	piperidine	14.6
	carbonate	0.021
	diethanolamine	0.032
phenyl	carbonate	0.95
	diethanolamine	1.01
	Tris	0.086
p-nitrophenyl	carbonate	27.5
	diethanolamine	85.7
	Tris	0.53
	acetate	0.03

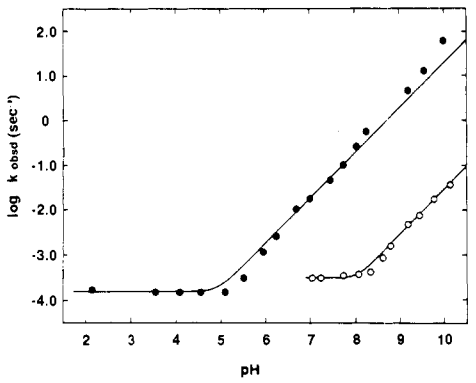


Figure 5. Plots of log k_{obsd} vs pH for release of *p*-nitrophenol from *p*-nitrophenyl 2-(carboxamido)phenylcarbonate (V) (●) and *p*-nitrophenyl 4-(carboxamido)phenylcarbonate (○) in H_2O at 30 °C, μ = 0.5 M with KCl.

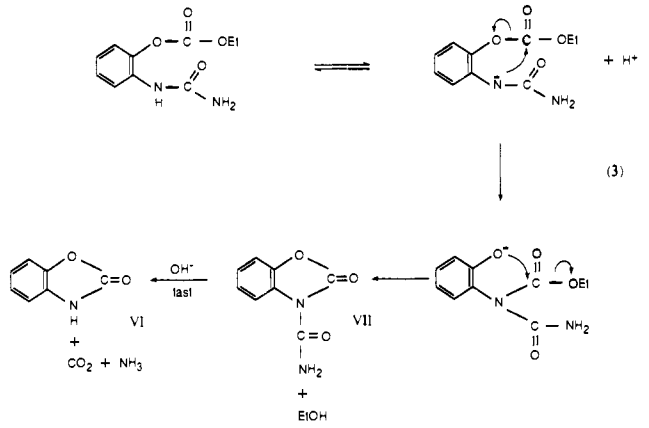
I–III, therefore, contain K_a. On the other hand, the rate constants (k_{OH}) for OH^--catalyzed hydrolysis of the 4-ureido-substituted esters reflect attack of OH^- on the ester.

Buffer effects in the first step of the reactions of I–III were small, although with some amine buffers (piperidine, ethylenediamine, diethanolamine, *N*-ethylmorpholine, Tris, and imidazole) increasing the buffer concentration at constant pH produced a small increase in k_{obsd}. For example, in the initial reaction of I, the highest concentration of total buffer (0.5 M) increased the rate by a factor of 10.3% with diethanolamine and 17.9% with Tris. Pronounced buffer catalysis was, however, observed with the corresponding 4-ureidophenyl esters. Second-order rate constants for these reactions are given in Table II. The intermediate in the reactions of II also reacted with strong buffer catalysis, k_B(piperidine) = 20.6 M^{-1} s^{-1} and k_B(*N*-ethylmorpholine) = 3.19×10^{-3} M^{-1} s^{-1}.

In Figure 5 is shown a plot of log k_{obsd} vs pH for release of *p*-nitrophenol from V at 30 °C in H_2O with μ = 0.5 M. Apparent hydroxide ion catalysis is observed at pH > 6 with $k_{OH} = 1.7 \times 10^5$ M^{-1} s^{-1}. In contrast, the k_{OH} for *p*-nitrophenol release from the analogous *p*-carboxamido derivative is 1.8×10^2 M^{-1} s^{-1} at 30 °C. With both the 2- and 4-substituted esters, the observed reaction at lower pH is pH independent and proceeds with very similar values of k_{obsd}. Therefore, the pH-independent reactions must be water-mediated hydrolysis. The final product in the hydrolysis of V was shown to be salicylamide.

Discussion

The cyclization of I occurs in two observable steps. The first reaction, in which phenol is released, proceeds with apparent OH^- catalysis, thereby showing that nucleophilic attack is by the anionic species of the neighboring ureido group. The second step has a sigmoidal pH–rate constant profile with an apparent pK_a of 8.9 (see Figure 1). This most likely indicates participation in the second step by the neighboring phenoxide ion liberated in the initial nucleophilic reaction (eq 3). The product of the second step was



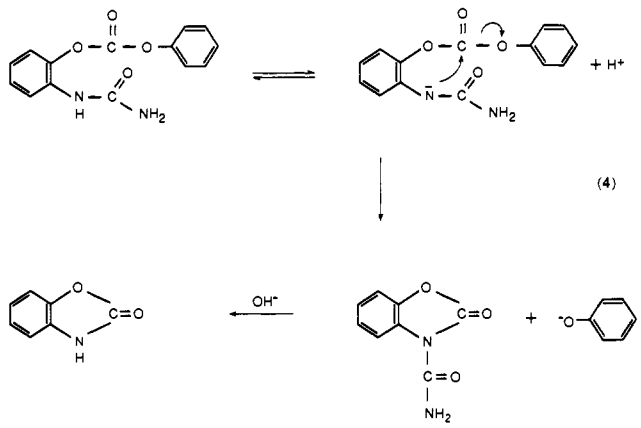
conclusively identified as benzoxazolinone (VI). From the qualitative and quantitative identity of the UV spectrum of the product with that of an authentic sample of benzoxazolinone,[13] it can be concluded that VI is the only product formed other than CO_2, NH_3, and ethanol.[14] The neighboring phenoxide ion is acting as a nucleophile in the second step in view of the fact that the reaction proceeds at nearly the same rate in D_2O as in H_2O. If the phenoxide ion was acting as a general base, partially abstracting a proton from a water molecule in the transition state, then the reaction would be considerably slower in D_2O than in H_2O.[6] Benzoxazolinone could only arise in the type of reaction depicted in eq 3. Thus, the initial nucleophilic attack is by the ureido nitrogen. Nitrogen attack in the first step is required to explain the formation of benzoxazolinone in the second step in a chemically reasonable manner. The initial reaction involves expulsion of phenoxide ion since phenoxide is much less basic than ethoxide, even though a more favorable ΔS^* would result from ethoxide departure.

In the cyclization reactions of II, phenol release is rapid and an intermediate is produced that subsequently is converted to benzoxazolinone in a slower OH^--catalyzed reaction. The cyclic compound VII was prepared synthetically and exhibited an identical UV spectrum and rate constants in benzoxazolinone formation as the product of the cyclization reaction. Consequently, the initial reaction must be proceeding with nucleophilic attack by nitrogen as in eq 4. In accord with this scheme, methylation of the *N*-phenyl nitrogen to give IV reduces the second-order rate constant (k_{OH}) for phenol release by approximately 1800-fold.[15] The apparent OH^- catalysis again indicates that nucleophilic attack is via an anionic species. The rate constant (k_{OH}) for the first step (nucleophilic attack) in the reaction of II is 10-fold larger than the corresponding rate constant for I because II breaks down into two molecules, whereas I simply rearranges. This should result in a more favorable ΔS^* for reaction of II than I. Note also that the k_{obsd} values for hydrolysis of the intermediate (VII) in the reaction of II are all at least 10-fold larger than those in the second reaction of I, thereby explaining why a third step (hydrolysis of

(13) Benzoxazolinone and *N*-methylbenzoxazolinone are very stable at the pH values studied. Hutchins, J. E. C.; Fife, T. H. *J. Am. Chem. Soc.* **1973**, *95*, 2282.

(14) Attack of OH^- may occur at either carbonyl group of VII.

(15) The second-order rate constant (k_{OH}) for phenol release from the *N*-methylated derivative IV (67 M^{-1} s^{-1}) represents an upper limit for intramolecular oxygen attack via a 7-membered-ring transition state.



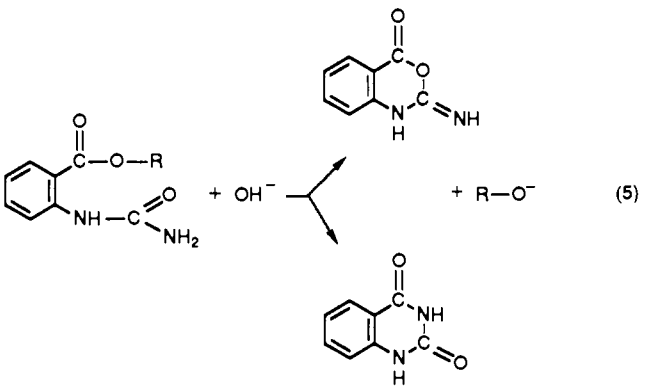
(4)

VII

N-carbamoylbenzoxazolinone) is not experimentally observed in the cyclization of I (see eq 3).

In comparison with the corresponding *p*-ureidophenyl derivatives, which hydrolyze with OH^- catalysis, the initial OH^--catalyzed phenol release from I–III is $>10^4$-fold faster. Thus, the neighboring ureido group is an efficient nucleophile in these reactions. The rate enhancements are comparable to those obtained in the intramolecular ureido group reactions of carboxylate esters.[7]

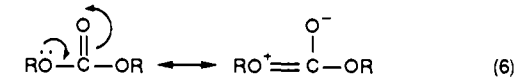
Bimolecular nucleophilic reactions of imidazolone or urea with carboxylate esters have not been observed.[16-18] Likewise, amides have little nucleophilicity in bimolecular reactions. However, in intramolecular reactions, neighboring amide groups are highly efficient nucleophiles.[19-22] These reactions generally occur via the anionic species,[19-22] and oxygen is an efficient nucleophile when attack by nitrogen is sterically precluded.[22] In a similar manner, a neighboring ureido group participates as a nucleophile in reactions of benzoate esters with various leaving groups.[7] In the reactions of the anionic species, both oxygen and nitrogen attack occurs (eq 5), depending upon the basicity of the leaving group.



(5)

With the methyl ester, a quinazoline (nitrogen attack) is formed exclusively, but in the case of the phenyl ester, 8% oxazine (oxygen attack) and 81% quinazoline are formed. With the *p*-nitrophenyl ester, the product is 58% oxazine. The carbonate diesters II and III with phenolic leaving groups cyclize 25–33-fold more rapidly than the carboxylate esters with the same leaving groups at 30 °C via exclusive nitrogen anion attack. A neutral species reaction with oxygen attack also takes place with the carboxylate phenolic esters at pH < 7, which is not detected with the carbonate diesters at pH > 3. Thus, there are significant kinetic and mechanistic differences in the cyclization reactions of these types of compounds.[23]

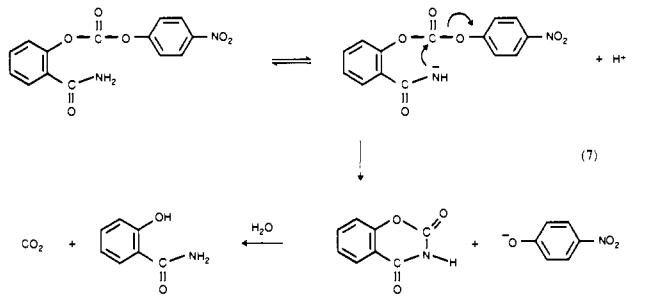
The phenoxy or alkoxy remaining group adjoining the carbonyl of carbonate diesters will exert an electron-withdrawing inductive effect that will make attack of the nucleophile easier but that will retard departure of the leaving group. The remaining group will also donate electrons through a resonance effect (eq 6), which



(6)

will deactivate the carbonyl, thereby hindering the attack step, but which will aid departure of the leaving group. The relative importance of these effects will depend upon the transition-state structure. In view of the reasonably small enhancing effect of changing the leaving group from phenol to *p*-nitrophenol in these reactions (18-fold), it is likely that nucleophilic attack by the negatively charged ureido group is rate limiting, i.e., there is little or no bond breaking in the critical transition state. This is supported by the nearly constant ratios of $k_{OH}(ortho)/k_{OH}(para) = 10^4$ for each leaving group. In this comparison, the second-order rate constant for the apparent OH^--catalyzed ureido nucleophilic reaction is compared with the second-order rate constant for OH^--catalyzed hydrolysis of the para derivative, and in the latter reaction, attack of OH^- would be expected to be rate determining.[6]

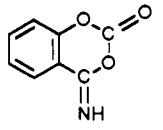
In the intramolecular nucleophilic reactions of I–III, nitrogen attack can proceed through a kinetically favored 5-membered-ring transition state, whereas oxygen attack would require a 7-membered-ring transition state. In these circumstances, nitrogen attack could be more favorable than oxygen attack solely on the basis of its steric advantage. With the *o*-carboxamido-substituted ester V, however, there is an equal opportunity from a steric standpoint for nitrogen and oxygen attack. The value of k_{OH} for *p*-nitrophenol release from V is 1000-fold larger than that of the analogous *p*-carboxamido-substituted ester. Thus, the neighboring amide group is participating in the reaction of V at pH > 6. The final product, which was isolated and identified, is salicylamide.[24] A prior cyclic intermediate was identified as 1,3-benzoxazine-2,4-dione (VIII). This shows that the initial nucleophilic attack must be by the amide nitrogen as in eq 7. Salicylamide would then



(7)

VIII

be formed by the slow hydrolysis of intermediate VIII. Initial oxygen attack would, however, produce a derivative (IX) that



IX

should hydrolyze rapidly. Nitrogen anion attack is favored over oxygen in the intramolecular amide group reactions of carbonate esters via a 6-membered-ring transition state.

(16) Caplow, M. *J. Am. Chem. Soc.* **1968**, *90*, 6795.
(17) Caplow, M.; Yager, M. *J. Am. Chem. Soc.* **1967**, *89*, 4513.
(18) Caplow, M. *J. Am. Chem. Soc.* **1965**, *87*, 5774.
(19) Behme, M. T.; Cordes, E. H. *J. Org. Chem.* **1964**, *29*, 1255.
(20) Shafer, J. A.; Morawetz, H. *J. Org. Chem.* **1963**, *28*, 1899.
(21) de Jersey, J.; Kortt, A. A.; Zerner, B. *Biochem. Biophys. Res. Commun.* **1966**, *23*, 745.
(22) Fife, T. H.; Pryzstas, T. J.; Pujari, M. P. *J. Am. Chem. Soc.* **1988**, *110*, 8157.

(23) Related carbamate esters have been found to give very large rate enhancements in intramolecular reactions (up to (2×10^8)-fold) in comparison with corresponding bimolecular reactions. Hutchins, J. E. C.; Fife, T. H. *J. Am. Chem. Soc.* **1973**, *95*, 3786. Fife, T. H.; Hutchins, J. E. C.; Wang, M. S. *J. Am. Chem. Soc.* **1975**, *97*, 5878.
(24) Salicylamide hydrolyzes slowly at 100 °C. Bruice, T. C.; Tanner, D. W. *J. Org. Chem.* **1965**, *30*, 1668.

While nucleophilic ureido or amido attack only takes place through the anionic species in reactions of carbonate diesters, a neutral species reaction can also occur in reactions of carboxylate esters with phenolic leaving groups. This must be a reflection of the relative activating and deactivating effects of the remaining group of a carbonate ester which clearly facilitates attack by the negatively charged nucleophile in a situation where attack by the anionic and neutral species is competitive. The Hammett ρ value reflecting the influence of electron withdrawal in the leaving group is generally much larger for attack of neutral nitrogen and oxygen nucleophiles on esters than is the case with negatively charged nucleophiles.[6-9] Therefore, nucleophilic attack by neutral nucleophiles very likely requires considerable C–O bond breaking in the transition state. As a consequence, the inductive effect of the carbonate ester remaining group, which is activating in the nucleophilic attack of anionic nucleophiles of high pK_a, becomes deactivating in reactions of neutral nucleophiles in which bond breaking is important. Therefore, a neutral species reaction is not competitive with attack of the ureido anion even at low pH.

That the cyclization reactions of the carbonate diesters proceed more readily via attack of the nitrogen anion than the oxygen anion must be due to the greater ease of attack of the nitrogen nucleophile[25,26] and/or the greater stability of a tetrahedral intermediate involving nitrogen toward reversion to reactants. Expulsion of a nitrogen anion from a tetrahedral intermediate (if one is formed) should be quite difficult, and nucleophilic attack would then be rate determining. Nucleophilic attack will be part of the rate-determining step if the reaction is concerted or if a tetrahedral intermediate partitions more readily to products than to reactants.

Biotin Reactions. Bruice and his co-workers[4,7,28] have suggested that in reaction of biotin with activated carbon dioxide the ureido oxygen is the initial nucleophile; *N*-carboxybiotin is then produced in a subsequent rearrangement. Guchhait et al.[3] have demonstrated that *N*-carboxybiotin will function as a carboxyl donor in enzymatic carboxylation reactions. From this it has been argued that the *N*-carboxylated species is probably the cofactor intermediate in the carboxylation reactions.[29,30] However, initial nucleophilic attack by the ureido oxygen on activated carbon dioxide followed by rearrangement cannot be excluded.[31] Kluger et al.[32] have suggested that the ureido oxygen is phosphorylated by ATP, which consequently enhances carboxylation of nitrogen by HCO_3^-. In this variation, it is again the ureido oxygen that is the initial nucleophile. On the basis of the present work, it can be concluded that nitrogen can serve as an efficient intramolecular nucleophile toward carbonate esters when the reaction proceeds via the anionic species, even at pH values less than 5. Nitrogen anion attack is also favored when there is no relative steric advantage for either nucleophile. The weak acidity of the neutral ureido NH function has been a concern with regard to postulations of nitrogen attack in biotin-catalyzed reactions.[33,34] However, it is clear that because of the great nucleophilicity of the nitrogen anion species in intramolecular reactions, its low concentration at neutral pH values or below is not a critical factor. There is no observable neutral species nucleophilic reaction with the carbonate diesters. Therefore, the high basicity and consequent nucleophilicity of the anionic species far outweigh in importance the much greater concentration of the neutral oxygen or nitrogen at pH values close to neutrality. Deprotonation of the ureido nitrogen occurs with sufficient rapidity that the anionic species could be involved in the enzymatic reactions.[35] The steric situation is clearly an important factor also. Efficient nitrogen anion attack will occur exclusively when a sterically favorable transition state is possible. However, in the enzymatic reactions the nucleophilic group of the biotin cofactor may depend on the manner in which the substrates are aligned in the enzyme active site. The initial nucleophile could, therefore, be different in the various enzymatic carboxylase reactions.

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

Registry No. I (isomer 1), 140678-00-8; I (isomer 2), 140678-05-3; II (isomer 1), 140678-01-9; II (isomer 2), 140678-06-4; III (isomer 1), 140678-02-0; III (isomer 2), 140678-07-5; IV, 140678-03-1; V (isomer 1), 140678-04-2; V (isomer 2), 140678-08-6; VI, 59-49-4; VII, 20844-72-8; *o*-ureidophenol, 1196-72-1; *o*-aminophenol, 95-55-6; *p*-ureidophenol, 1566-41-2; *p*-aminophenol, 123-30-8; ethyl chloroformate, 541-41-3; phenyl chloroformate, 1885-14-9; *p*-nitrophenyl chloroformate, 7693-46-1; *o*-(methylamino)phenol, 611-24-5; *o*-(*N*-methylureido)phenol, 87271-56-5; salicylamide, 65-45-2; 4-hydroxybenzamide, 619-57-8; biotin, 58-85-5.

(25) The factors of possible importance in nucleophilic attack at a carbonyl group have been previously discussed. Jencks, W. P. *Catalysis in Chemistry and Enzymology*; McGraw-Hill: New York, 1969; pp 78–111.

(26) Since inductive electron withdrawal from the carbonyl group appears to be a factor of importance in reactions of carbonate diesters, it can be inferred that there is greater net positive charge on the carbonyl carbon than with a carboxylate ester having the same leaving group. On this basis, a nitrogen anion could be somewhat more nucleophilic than oxygen toward a carbonate diester relative to a carboxylate ester.[27]

(27) In reactions at saturated carbon, with which polarizability effects are of importance, neighboring amide group reactions take place via attack of the oxygen anion. Zioudrou, C.; Schmir, G. L. *J. Am. Chem. Soc.* **1963**, *85*, 3258.

(28) Hegarty, A. F.; Bruice, T. C.; Benkovic, S. J. *Chem. Commun.* **1969**, 1173. They also consider reaction of an enol.

(29) Wood, H. G. *Trends Biochem. Sci.* **1976**, *1*, 4.

(30) Wood, H. G.; Barden, R. E. *Ann. Rev. Biochem.* **1977**, *46*, 385.

(31) In fact, *O*-carboxybiotin cannot be conclusively ruled out as the carboxyl donor to acceptor molecules. Even though the *N*-carboxy derivative might be thermodynamically more stable, the reverse rearrangement to the *O*-carboxylated species would provide an intermediate that should be kinetically much more reactive. Therefore, the transfer reaction could occur via that species.

(32) (a) Kluger, R.; Davis, P. P.; Adawadkar, P. D. *J. Am. Chem. Soc.* **1979**, *101*, 5995. (b) Kluger, R. *Chem. Rev.* **1990**, *90*, 1151.

(33) The pK_a of the neutral ureido group should be 10 or higher.

(34) Mildvan, A. S.; Fry, D. C. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1987**, *59*, 241.

(35) (a) Perrin, C. L.; Dwyer, T. J. *J. Am. Chem. Soc.* **1987**, *109*, 5163. (b) Fry, D. C.; Fox, T. L.; Lane, M. D.; Mildvan, A. S. *J. Am. Chem. Soc.* **1985**, *107*, 7659.