Acyl Transfer Reactions from and to the Ureido Functional Group. III. The Mechanism of Intramolecular Nucleophilic Attack of the Ureido Functional Group upon Acyl Groups

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Abstract: Intramolecular nucleophilic displacement by the ureido (and substituted ureido) functional group at the carbonyl carbon of esters and amides of *o*-ureidobenzoic acids has been investigated. Kinetic studies plus product analysis have led to the assignment of rate constants for O and N attack (by both the ionized and un-ionized ureido functional group). The dependence of rate and mode of anchimeric participation have been related to the basicity of the ureido group, the electron density of the benzoate moiety, and more completely to the nature of the leaving group. For *o*-(H_2NCONH)-C_6H_4CO-X the following results have been obtained: (a) when X^- represents a strong base (*i.e.*, $-O^-$, $-NH_2$, $-N(CH_3)_2$, $-OCH_3$, and $-OCH_2CF_3$) anchimeric participation is through the terminal ureido nitrogen as an anionic species to provide as the sole product the quinazoline **6**; (b) as the basicity of X^- decreases, anchimeric participation of the anionic ureido group occurs not only through N to give **6**, but increasingly through O to give the benzoxazine **4** ($-SCH_3$ and meta- and para-substituted phenols); and (c) for the best leaving groups anchimeric participation by O of the undissociated ureido functional group to give **4** becomes of increasing importance (meta- and para-substituted phenols). The relative sensitivities of the three mechanisms when phenolate anions represent the leaving group are for (a) $\log k_{rate} = -0.28\,pK_{phenol} + 6.6$; for (b) $\log k_{rate} = -0.74pK_{phenol} + 10.0$; and for (c) $\log k_{rate} = -0.87\,pK_{phenol} + 4.74$. Monomethylation of either ureido nitrogen has little influence on the rate constant associated with mechanism a though, expectedly, dimethyl substitution of the terminal nitrogen prevents reaction. The possibility of amide anion rather than ureido anion acting as an intramolecular nucleophile to displace $-NH_2$ from 2-ureidobenzamide **16** to provide the quinazoline **6** has been investigated by employing **16** labeled at the terminal N with ^{15}N. Mass spectral analysis of the product **6** showed that ~90 and ~100% of nucleophilic attack was by the ureido group under basic and acidic conditions, respectively. Under neutral conditions (H_2O) or with refluxing pyridine and pyrolysis the ureido nitrogen was eliminated. Arguments are presented in support of the cyclization of *o*-ureidobenzoate dianion occurring through ureido anion attack upon the *o*-carboxylate anion. The rate constant for this reaction is *ca.* 10^3 below that anticipated from the pK_a of the proposed leaving group and this is suggested to be due to the electrostatic barrier to attack of an anion upon a carboxylate anion. The propinquity effect (*i.e.*, $k_{intra}/k_{intermolecular}$) has been estimated at 10^8 *M* for participation of the neutral ureido group and at a minimum of 10^5 *M* for anionic ureido attack.

Biotin is required as a cofactor in a number of enzymatic carboxylation reactions (as examples: pyruvate + CO_2 → oxaloacetate;[3] acetyl-CoA + CO_2 → malonyl-CoA;[4] and β-methylcrotonyl-CoA + CO_2 → β-methylglutaconyl-CoA[5]). Carbon dioxide, activated in some little understood manner by ATP, is transferred to the imidazolone moiety of enenzyme-bound biotin and thence to the substrate to affect the carboxylation reaction.[6] Enzyme-bound biotin thus acts as a nucleophilic catalytic species in a double displacement reaction. The nucleophilicity of the ureido function of the imidazolone portion of biotin toward an acyl carbonyl group (activated CO_2 may be conceived as X-CO-OY) is, therefore, of fundamental importance in the understanding of the biotin-dependent carboxylation reactions. All attempts, to date, to realize any nucleophilicity of imidazolone or urea to acyl carbonyl groups, even when highly activated, have been unsuccessful.[7] Amides also exhibit little or no nucleophilicity toward the acyl carbonyl group in bimolecular reactions. However, in intramolecular displacement reactions the amide anion is one of the most effective nucleophiles known.[8] The general structural resemblance between the amide and ureido functional groups suggests that propinquity may well play an important role also in determining the nucleophilicity of the latter.

The present study deals with intramolecular displacement reactions of the ureido (and substituted ureido) group upon the acyl carbonyl moiety of *o*-ureidobenzoic acids and esters and amides derived therefrom. Our objectives have been to ascertain not only the dependence of the facility of the reaction upon the electron density of the acyl carbonyl and the basicity of the ureido functional group, but also the dependence of the mode of ureido participation (O *vs.* N attack by the ionized and un-ionized ureido group) upon the nature of the leaving group. The latter factor is of likely significance in understanding the mode of action of biotin since it cannot be stated with certainty which position of biotin acts as the nucleophilic center.[9,10]

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(3) R. J. Winzler, D. Burk, and V. DuVigneaud, *Arch. Biochem. Biophys.*, **5**, 25 (1944); J. L. Stokes, A. Larsen, and M. Gunness, *J. Bacteriol.*, **54**, 219 (1947); H. A. Lardy, R. L. Potter, and C. A. Elvehjem, *J. Biol. Chem.*, **169**, 451 (1947); W. Shive and L. L. Rogers, *ibid.*, **169**, 453 (1947); H. C. Lichtstein and W. W. Umbreit, *ibid.*, **170**, 329 (1947).

(4) S. J. Wakil, E. B. Titchener, and D. M. Gibson, *Biohem. Biophys. Acta*, **29**, 225 (1958); S. J. Wakil and D. M. Gibson, *ibid.*, **41**, 122 (1960); M. Waite and S. J. Wakil, *J. Biol. Chem.*, **237**, 2750 (1962).

(5) F. Lynen, *Proc. Int. Symp. Enzyme Chem., 1957*, 57 (1958).

(6) For a review see: T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms," Vol. II, W. A. Benjamin, New York, N. Y., Chapter 11.

(7) M. Caplow, *J. Amer. Chem. Soc.*, **90**, 6796 (1968); M. Caplow and M. Yager, *ibid.*, **89**, 4513 (1967); and M. Caplow, *ibid.*, **87**, 5774 (1965).

(8) M. T. Behme and E. H. Cordes, *J. Org. Chem.*, **29**, 1255 (1964).

Experimental Section

Materials. Methyl 2-Ureidobenzoate. Methyl anthranilate (15.2 g, 0.10 mol) was vigorously stirred into 200 ml of glacial acetic acid–water (1:1) at room temperature and cooled to 10°. Potassium cyanate (8.1 g, 0.10 mol), dissolved in 20 ml of water, was added slowly. The methyl 2-ureidobenzoate which precipitated (in near quantitative yield) was recrystallized by dissolution in absolute methanol at 30°, addition of charcoal, followed by filtration and cooling to 0°; the resultant product had mp 175–177° dec (lit.[11] mp 177° dec). *2*-Ureidobenzoic acid, mp 170–171° (lit.[12] mp 170–171°), and 2-ureidobenzamide, mp 180–182° (when heated rapidly to 170°) (lit.[13] mp 184–185°), were similarly prepared from anthranilic acid and 2-aminobenzamide, respectively.

Methyl 4-Ureidobenzoate. 4-Aminobenzoic acid (2.0 g, 0.015 mol) was dissolved at 40° in 70 ml of water containing 2 ml of acetic acid. On cooling to room temperature an equimolar amount (1.2 g) of potassium cyanate, dissolved in 10 ml of water, was added dropwise. The 4-ureidobenzoic acid which precipitated was recrystallized from methanol–water and had mp >360°.[14] The acid (1.0 g, 0.0055 mol) was converted to its methyl ester by treatment (in ether solution) with an excess of diazomethane, prepared from *N*-nitrosomethylurea (1.03 g, 0.01 mol). Methyl 4-ureidobenzoate had mp 188–190°. *Anal.* Calcd for $C_9H_{10}N_2O_3$: C, 55.66; H, 5.19; N, 14.43. Found: C, 55.51; H, 5.27; N, 14.31.

2′,2′,2′-Trifluoroethyl 2-Ureidobenzoate. To a solution of 2 g (0.012 mol) of isatoic anhydride in 40 ml of warm (*ca.* 40°) dioxane was added 1.22 g (0.012 mol) of 2,2,2-trifluoroethanol, dissolved in 5 ml of dioxane. Dry powdered potassium hydroxide (25 mg) was added as catalyst and the solution was slowly heated (over 0.5 hr) to 100°. After a further 0.5 hr at 100°, 2′,2′,2′-trifluoroethyl 2-aminobenzoate was precipitated by addition of 150 ml of water to the cooled dioxane solution; crystallization from methanol gave 1.2 g (49%), mp 43–44° (lit.[15] mp 44°). The 2-amino ester was converted to the 2-ureido ester by treatment with an equimolar amount of potassium cyanate in 50:50 acetic acid–water as described above for methyl 2-ureidobenzoate. 2′,2′,2′-Trifluoroethyl 2-ureidobenzoate had mp 178–180° on crystallization from methanol below 50°. *Anal.* Calcd for $C_{10}H_9F_3N_2O_3$: C, 45.81; H, 3.46; N, 10.69. Found: C, 49.95; H, 3.36; N, 11.25. In a similar manner, using isatoic anhydride and methyl mercaptan as starting materials, **methylthio 2-ureidobenzoate**, mp 160–162°, was prepared. *Anal.* Calcd for $C_9H_{10}N_2O_2S$: C, 51.41; H, 4.79; N, 13.33. Found: C, 51.86; H, 5.14; N, 14.08.

Methyl 2-(*N′*-Methylureido)benzoate. A solution of 2.0 g (0.013 mol) of methyl anthranilate and 0.85 g (0.015 mol) of methyl isocyanate was refluxed for 2 hr in 50 ml of dry benzene. On evaporation of the solvent the residue was dissolved in methanol at 40°, and filtered, and cooled to 0°. After two further recrystallizations of the precipitated product from hexane, the ureido benzoate had mp 113°. *Anal.* Calcd for $C_{10}H_{12}N_2O_3$: C, 57.68; H, 5.81; N, 13.46. Found: C, 57.57; H, 5.91; N, 13.30.

Methyl 2-(*N′*,*N′*-Dimethylureido)benzoate. To a solution of 2.0 g of isatoic anhydride (0.012 mol) in 50 ml of dioxane was added 9.0 g of 25% w/w aqueous dimethylamine solution (*ca.* 0.05 mol) and the solution stirred at room temperature for 30 min. Water (50 ml) was added and 2-(*N′*,*N′*-dimethylureido)benzoic acid was precipitated by acidification of the solution with dilute HCl. On crystallization from MeOH–H_2O, it had mp 146–148° (lit.[15] mp 148°). The acid (1.0 g, 0.005 mol) was converted to the methyl ester by treatment with an excess of diazomethane (prepared from 0.77 g (0.010 mol) nitrosomethylurea) in ether solution at room temperature. Methyl 2-(*N′*,*N′*-dimethylureido)benzoate had mp 88–89° (from MeOH). *Anal.* Calcd for $C_{11}H_{14}N_2O_3$: C, 59.44; H, 6.35. Found: C, 59.25; H, 6.67.

Methyl 2-(*N*-Methylureido)benzoate. Methyl *N*-methylanthranilate was prepared by the method of Staiger and Miller.[5] The *N*-methylamino ester (1.0 g, 0.006 mol) was dissolved in 40 ml of a 1:1:1 mixture of dioxane, water, and acetic acid and slowly cooled to 5°; a small amount of undissolved material was filtered off. Potassium cyanate (0.64 g, 0.008 mol) in 5 ml of water was added. The precipitated ureido ester was filtered off and water (100 ml) was added to the filtrate which was extracted with ether. Evaporation of the dried ether extracts yielded further crude ureido ester (total yield = 760 mg, 62%). The methyl 2-(*N*-methylureido)benzoate (MeOH, maintained below 50°) had mp 151°, resolidified immediately, and remelted at >300°. *Anal.* Calcd for $C_{10}H_{12}N_2O_3$: C, 57.68; H, 5.81; N, 13.46. Found: C, 57.57; H, 5.91; N, 13.30.

2-(*N′*,*N′*-Dimethylureido)benzamide. To a solution of 0.85 g (0.0062 mol) of 2-aminobenzamide in pyridine (10 ml) was added 0.70 g (0.0065 mol) of dimethylcarbamoyl chloride. The solution was heated to 60° for 15 min and then, on cooling to room temperature, allowed to stand at room temperature for 48 hr. The solvent was evaporated *in vacuo* and the residual solid recrystallized several times from methanol–water. The 2-(*N′*,*N′*-dimethylurea)benzamide obtained had mp 175°. *Anal.* Calcd for $C_{10}H_{13}N_3O_2$: C, 57.96; H, 6.32; N, 20.28. Found: C, 57.88; H, 6.60; N, 20.20.

2-Ureido-*N*,*N*-dimethylbenzamide. A solution of 1.0 g (0.0037 mol) of 2-amino-4,5-benzo-6-oxo-1,3-oxazine (prepared by the method of Lempert and Doleschall[16]) in 20 ml of dioxane was treated with an excess of dimethylamine gas (prepared by dropping 10 ml of 25% aqueous dimethylamine solution on KOH pellets). The solution was stirred at room temperature for 1 hr and the solvent evaporated. Crystallization from dioxane gave the amide (0.32 g, 42%), mp 175°. *Anal.* Calcd for $C_{10}H_{13}N_3O_2$: C, 57.96; H, 6.32; N, 20.28. Found: C, 57.88; H, 6.51; N, 20.07.

2-Ureido-*N*-phenylbenzamide was prepared by a literature procedure[17] and had mp 276–278°, after preliminary softening at 198° (lit.[17] mp 278–280° dec).

Methyl 2-Ureido-5-chlorobenzoate. A solution of 5 g (0.025 mol) of 5-chloroisatoic anhydride in 100 ml of dry MeOH (containing 100 mg of dry powdered KOH catalyst) was refluxed for 30 min. The solution was filtered while hot and on cooling and addition of water (50 ml), methyl 2-amino-5-chlorobenzoate was obtained (4.6 g, 88%), mp 66–67° (ex, MeOH–H_2O). *Anal.* Calcd for $C_8H_8ClNO_2$: C, 51.76; H, 4.34; N, 7.55. Found: C, 51.73; H, 4.45; N, 7.23. Using a procedure similar to that outlined in the preparation of methyl 2-ureidobenzoate (above), the amino compound was converted to methyl 2-ureido-5-chlorobenzoate, mp 198–200°. *Anal.* Calcd for $C_9H_9ClN_2O_3$: C, 47.27; H, 3.97; N, 12.25. Found: C, 47.40; H, 4.06; N, 12.10.

Methyl 2-Ureido-4-nitrobenzoate. A solution of 6.3 g (0.035 mol) of 4-nitroanthranilic acid in 40 ml of water containing 2.3 g (0.035 mol) of potassium hydroxide (85%) was added dropwise over 30 min to a slurry of cyanogen bromide [prepared from 7.2 g (0.045 ml) of bromine and 5.2 g (0.08 mol) of potassium cyanide, each dissolved in 20 ml of water] at 0°. The 4′-nitro-2-amino-4,5-benzo-6-oxo-1,3-oxazine formed was filtered off and washed thoroughly with water; it had mp 320–322°. The oxazine (1.0 g, 0.0048 mol) was stirred in 50 ml of dry dioxane and dry HCl passed through the solution. The precipitated HCl salt was filtered off and stirred with 50 ml of methanol at room temperature for 2 hr. The undissolved material was filtered off and water (50 ml) was added to the filtrate to precipitate methyl 2-ureido-4-nitrobenzoate (0.48 g, 42%), mp 206–208° dec (from MeOH). *Anal.* Calcd for $C_9H_9N_3O_5$: C, 45.19; H, 3.79. Found: C, 44.47; H, 3.73.

Phenyl 2-Ureidobenzoate. To a solution of 5 g (0.031 mol) of isatoic anhydride in 100 ml of dioxane at 50° was added 2.9 g (0.031 mol) of phenol and 100 mg of powdered KOH. The solution was slowly heated to 100° until evolution of CO_2 ceased (40 min). On cooling, water (200 ml) was added and the precipitated phenyl 2-aminobenzoate (4.7 g, 72%) was recrystallized from methanol–water to mp 71–72° (lit. mp 70°). Using the method outlined above in preparation of methyl *N*-methylureidobenzoate, this amino ester was converted to phenyl 2-ureidobenzoate, mp 156° dec. *Anal.* Calcd for $C_{14}H_{12}N_2O_3$: C, 65.61; H, 4.72; N, 10.93. Found: C, 66.02; H, 4.85; N, 11.36. The same general method was used to prepare the following 2-amino and 2-ureido esters.

4′-Chlorophenyl 2-aminobenzoate had mp 79–80° (40–60° petroleum ether). *Anal.* Calcd for $C_{13}H_{10}ClNO_2$: C, 63.04; H, 4.07; N, 5.65. Found: C, 63.16; H, 3.78; N, 5.78.

4′-Chlorophenyl 2-ureidobenzoate exhibited mp 157° (MeOH). *Anal.* Calcd for $C_{14}H_{11}ClN_2O_3$: C, 57.84; H, 3.81; N, 9.64. Found: C, 56.56; H, 4.01; N, 10.34.

(9) T. C. Bruice and A. F. Hegarty, *Proc. Natl. Acad. Sci. U. S.*, **65**, 805 (1970).
(10) A. F. Hegarty and T. C. Bruice, *J. Amer. Chem. Soc.*, **92**, 6561 (1970).
(11) M. Taylor and G. Scatchard, *ibid.*, **41**, 2052 (1919).
(12) R. P. Staiger and E. C. Wagner, *J. Org. Chem.*, **18**, 427 (1953).
(13) W. A. Jacobs and M. Heidelberger, *J. Amer. Chem. Soc.*, **39**, 2437 (1917).
(14) T. L. Davies and K. C. Blanchard, *ibid.*, **51**, 1790 (1929).
(15) R. P. Staiger and E. B. Miller, *J. Org. Chem.*, **24**, 1214 (1959).
(16) K. Lempert and G. Doleschall, *Monatsh. Chem.*, **95**, 950 (1964).
(17) G. Doleschall and K. Lempert, *ibid.*, **95**, 1068 (1964).

4'-Nitrophenyl 2-aminobenzoate had mp 108–110° (lit.[15] 109°).

4'-Nitrophenyl 2-ureidobenzoate had mp 140–145° (hexane). *Anal.* Calcd for $C_{14}H_{11}N_3O_5$: C, 55.81; H, 3.68; N, 13.95. Found: C, 55.46; H, 4.01; N, 13.47.

3'-Nitrophenyl 2-aminobenzoate showed mp 106° (hexane). *Anal.* Calcd for $C_{13}H_{10}N_2O_2$: C, 60.46; H, 3.90; N, 10.84. Found: C, 60.41; H, 4.23; N, 10.78.

3'-Nitrophenyl 2-ureidobenzoate had mp 130–133° (hexane). *Anal.* Calcd for $C_{14}H_{11}N_3O_5$: C, 55.81; H, 3.68; N, 13.95. Found: C, 56.17; H, 4.28.

Methyl 2-(*N'*-Phenylureido)benzoate. A solution of 7.5 g (0.05 mol) of methyl anthranilate was refluxed in 50 ml of dry ether with 5.9 g (0.05 mol) of phenyl isocyanate for 2 hr. The solution was allowed to stand at room temperature overnight, the volume of the solution halved by evaporation, and the precipitated methyl 2-(*N'*-phenylureido)benzoate (*ca.* quantitative yield) collected, mp 142–144° (MeOH) (lit.[16] mp 143–144°). The following *N'*-(substituted phenyl)-*N*-(2-carboxymethyl)ureas were similarly prepared.

Methyl 2-(*N'*-*p*-tolylureido)benzoate showed mp 150° (MeOH). *Anal.* Calcd for $C_{16}H_{16}N_2O_3$: C, 67.58; H, 5.67; N, 9.85. Found: C, 67.67; H, 5.64; N, 9.96.

Methyl 2-(*N'*-*p*-chlorophenylureido)benzoate exhibited mp 170° (MeOH). *Anal.* Calcd for $C_{15}H_{13}ClN_2O_3$: C, 59.12; H, 4.29; N, 9.20. Found: C, 59.87; H, 3.90; N, 9.32.

Methyl 2-(*N'*-*p*-nitrophenylureido)benzoate had mp 230–232° (MeOH). *Anal.* Calcd for $C_{15}H_{13}N_3O_5$: C, 57.14; H, 4.16; N, 13.33. Found: C, 57.24; H, 4.05; N, 13.17.

***N*-(*o*-Benzamido)-*N'*-[^{15}N]-urea and Cyclization Studies.** Potassium [^{15}N]-cyanate was prepared by mixing 440 mg of dry potassium carbonate and 500 mg of [$^{15}N_1$]-urea (50 atom %, purchased from Internal Chemical and Nuclear Corporation) in a crucible over an open gas flame.[18] The yield was quantitative. This was converted to *N*-(*o*-benzamido)-*N'*-[^{15}N]-urea by the method of Jacobs and Heidelberger.[13] This 2-ureidobenzamide had mp 182–184° dec (lit.[13] 184–185° dec) on crystallization from methanol. It was shown to contain 33.5 atom % ^{15}N at the terminal nitrogen of the ureido group (by comparison with an unlabeled reference) by mass spectral analysis. Mass spectra were obtained using an AEI Model MS 902 double focussing mass spectrometer. A direct inlet system was used with usually 35-eV electron beam energy and source temperature in the region 100–120°. A minimum of four traces of intensity *vs.* *m/e* (in both directions) on a strip chart recorder were used to calculate the relative per cent ^{15}N enrichment in a given compound. In all cases, clear parent peaks were distinguishable.

The cyclization of *N*-(*o*-benzamido)-*N'*-[^{15}N]-urea to 3-[^{15}N]-2,4-(1*H*,3*H*)-quinazolinedione was studied under the following conditions. The atom per cent ^{15}N found in the cyclic product (relative to the starting material) is listed in the Results.

(a) A solution of 50 mg of (labeled) 2-ureidobenzamide in 2 ml of 1 *N* KOH was maintained at 50° for 5 min. On cooling, the cyclic product precipitated. This was redissolved by the addition of water (10 ml) and reprecipitated by neutralization with dilute HCl. The quinazoline had mp 350–354°.

(b) A solution of 30 mg of 2-ureidobenzamide was dissolved at 50° in 2 ml of 1 *N* HCl. The quinazoline (which precipitated almost immediately) was collected after 5 min and washed thoroughly with water.

(c) In 2 ml of dry pyridine, 30 mg of the 2-ureidobenzamide was refluxed for 10 hr. On removal of the pyridine by evaporation, the quinazoline was thoroughly washed with water.

(d) The 2-ureidobenzamide (30 mg) was heated in the absence of solvent at 180° for 30 min and analyzed directly.

(e) A solution of 30 mg of the 2-ureidobenzamide was refluxed for 10 hr in 2 ml of unbuffered aqueous solution. The quinazoline precipitated on cooling.

(f) The cyclization on electron impact was also studied (at 35 eV, source = 121°) by comparing the relative intensities of peaks at mass no. 179, 180 with those at 162, 163 (relative to unlabeled 2-ureidobenzamide).

All inorganic materials used in the preparation of solutions for kinetic experiments were Reagent grade and used without further purification. The water used was deionized, then twice distilled from all-glass apparatus. Glycine (Fischer Reagent grade) and urea (Baker and Adamson Reagent grade) were used directly.

Kinetic Measurements. All kinetic experiments were carried out in aqueous solution at 30°. Ionic strength was maintained at 1.0 by the addition of potassium chloride as necessary. In all cases the rates of cyclization were followed spectrophotometrically. Preliminary repetitive scans of the region 380–220 nm established the most suitable wavelength(s) to study the reactions. Since the product quinazoline has a pK_a (with a large spectral change between the neutral and ionized forms) in the region often used to study pH–rate profiles, it was often found convenient to use several different wavelengths, *e.g.*, 255 nm with pH < 10, 265 nm with pH > 9 for methyl 2-ureidobenzoate to follow the OD change in a given cyclization. In all cases similar rate constants were calculated from data obtained at the different wavelengths.

Except in those experiments in which buffer effects were specifically investigated, pH–rate profiles were obtained without the addition of external buffers. pH was maintained either by hydroxide ion (at high pH) or by the use of a spectrophotometric–pH-stat assembly described elsewhere.[19] In the latter case a Cary 15 spectrophotometer was fitted with a 25-ml cell constructed so that a Radiometer pH-stat (consisting of Type PHM 26 pH meter, Type TT 11b titrator, Type SBR 2C titrigraph, and a Type ABU 1C autoburette) maintained constant pH by the addition of small aliquots of potassium hydroxide solution. At high pH, either a Gilford Model 2000 spectrophotometer (3-ml cuvettes) or a Durrum-Gibson Model 13001 stopped-flow spectrophotometer was used, and pH was measured both before and after a kinetic run using a Radiometer pH meter Type PHM 22 equipped with a PHA 630 scale expander.

Either dioxane or acetonitrile (Matheson Coleman and Bell Spectroquality) was used to make stock solutions of the substrates. The final concentration of the organic component was always <1% [except in the case of *N*-(*p*-nitrophenyl)-*N'*-(*o*-carboxymethylphenyl)urea where 3% dioxane was required to assure a solution].

The pseudo-first-order rate constants were calculated from the slopes of plots of log $(OD_t - OD_\infty)$ *vs.* time (t). In all cases a minimum of four (and more usually six) rate determinations were made for a given substrate covering a minimal pH range of 2 units. In one case (the cyclization of methyl 2-ureidobenzoate) pH's from 7 to 13 were studied [with observed pseudo-first-order rate constants (k_{obsd}) in the range 10^{-4}–10^{2} sec^{-1}]. Except where noted, plots of log k_{obsd} *vs.* pH were linear with slope = +1.0. Such plots yielded the second-order rate constants (k_2) quoted

$$k_2 = k_{obsd}/(K_w/a_{H^+})$$

i.e., $k_2 = k_{obsd}$ at pH = pK_w.

pK_a Determinations. pK_a's were measured in the same cell[19] (described above) and under the same conditions [30°, μ = 1.0 (KCl)] used for the kinetic experiments. Both pH *vs.* change in OD and pH *vs.* milliliter of base added data were obtained in each determination. pK_a's were calculated by comparison with theoretical curves, both methods giving identical results.

Product Analysis. Many of the cyclizations described in this study have been shown to give, on a preparative scale, near quantitative yields in alkaline solution of 2,4-(1*H*,3*H*)-quinazolinedione, *e.g.*, cyclization of methyl 2-ureidobenzoate[11] (also ethyl, *n*-propyl etc. ester[17]), 2-ureidobenzamide[13] (and other amides[17]), and of 2-ureidobenzoic acid.[20]

The following technique was used to analyze the products formed during a kinetic experiment, showing that in the alkaline region 2,4-(1*H*,3*H*)-quinazolinedione was the principal (and usually the sole) product. On completion of the cyclization, the pH of the solution was adjusted to *ca.* 5. Spectrophotometric scans of the region 380–240 nm were recorded as the pH was increased stepwise (to ~11). The resultant spectra were compared with those of an authentic sample of the quinazoline, and the pK_a calculated from the data compared with that of the quinazoline (9.60). For those compounds with poorer leaving groups, this method generally showed 100% quinazoline formation.

A modified technique was used to estimate relative amounts of attack by ureido-*O* (giving 2-amino-3,4-benzo-6-oxo-1,3-oxazine) and -*N* [giving 2,4-(1*H*,3*H*)-quinazolinedione]. (a) The initial cyclization was allowed to go to completion at a given pH. (b) The pH was adjusted rapidly to 9.9 and the change in OD at 265 nm recorded. At this pH cleavage of the oxazine occurs (to form 2-ureidobenzoic acid)[10] with k_{obsd} *ca.* 4×10^{-3} sec^{-1}. The OD change resulting on hydrolysis of an authentic sample of the oxazine was also recorded at 265 nm at pH 9.9. Comparison of these

(18) A. Scattergood, *Inorg. Syn.*, **2**, 86 (1946).

(19) J. R. Maley and T. C. Bruice, *Anal. Biochem.*, **34**, 275 (1970).

(20) N. A. Lange and F. E. Sheibley, "Organic Syntheses," Coll. Vol. II, Wiley, New York, N. Y., 1943, p 79.

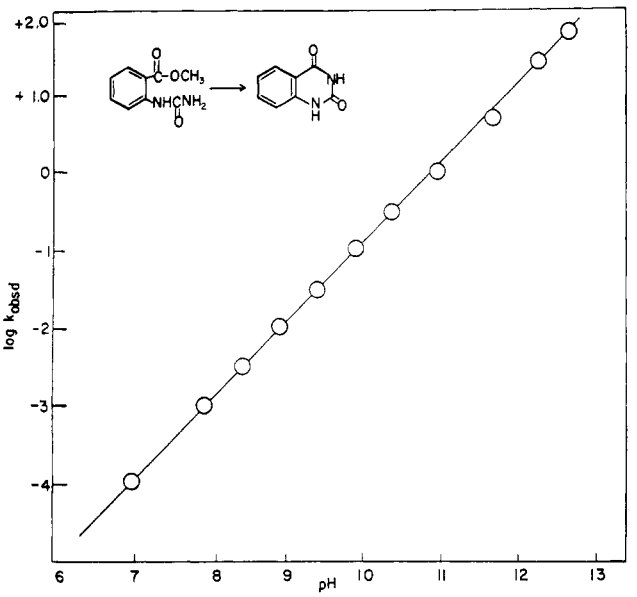


Figure 1. pH–log k_{obsd} profile for **5** → **6** (30°, μ = 1.0, H_2O; rate constants in units of sec^{-1}).

results permitted the calculation of the amount (and hence the per cent) of the oxazine formed in the original cyclization. (c) The pH was adjusted to ~5 and repetitive spectral scans recorded as the pH was raised to ~11. The difference in OD between the neutral and anionic forms of the quinazoline (usually at 340 nm) permitted the calculation of the per cent N-attack product. The other product present at this stage, 2-ureidobenzoic acid, does not have a pK_a in this region and neither does it recyclize. With substituted phenoxide leaving groups, an added complication was the spectral change of the phenoxide–phenol equilibrium. This was overcome by measuring the OD change of the quinazoline as the pH varied either (i) at a wavelength where neither the phenoxide or phenol absorb, *e.g.*, 340 nm when phenol or *p*-chlorophenol was present, or (ii) at an isosbestic point of the phenol, *e.g.*, 347 nm in the presence of *p*-nitrophenol, 266 nm for *m*-nitrophenol. The relative amounts of the products calculated by this method follow in the Results.

Results

The kinetics of cyclization of a number of 2-ureidobenzoates and related compounds have been investigated in aqueous solution (μ = 1.0) at 30°. In each case the observed rate constants could be described in terms of the empirical paths of eq 1; generally only the

$$\underbrace{\mathrm{E} \underset{+\mathrm{H}^-}{\overset{-\mathrm{H}^+,\,K_{a1}}{\rightleftharpoons}} \mathrm{E}^-}_{\text{products}} \qquad \begin{matrix} \downarrow k_0 & \downarrow k_2' \end{matrix} \tag{1}$$

$$k_{obsd} = k_0 + k_2' \frac{K_{a_1}}{K_w}[HO^-] \tag{2}$$

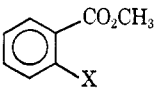
$$= k_0 + k_2[HO^-]$$

second-order (k_2) term in eq 2 is detectable. Each compound showed a region where the observed rate constants were proportional to the hydroxide ion concentration. The reactivities are expressed in terms of k_2, the observed rate constant when [HO^-] = 1.0. This procedure was necessitated since K_{a_1} was not determined; listing k_2 has the advantage that direct comparison with other hydroxide-catalyzed reactions is possible.

Data for the cyclization of the compound chosen as standard, methyl 2-ureidobenzoate, are shown in Figure 1. The plot of log k_{obsd} *vs.* pH is linear with slope = +1.0 over the entire range studied. The spontaneous term k_0 did not contribute (*i.e.*, $k_0 < 10^{-5}$ sec^{-1}); neither was pK_{a_1} approached at the highest pH studied (12.7).

Substituent Effects on k_2. (a) *N*-Methyl. The values of k_{obsd} obtained at various pH's together with the derived constants k_2 are listed in Table I for various

Table I. Observed Pseudo-First-Order and Second-Order (k_2) Rate Constants for Cyclization or Hydrolysis of Methyl ortho-Substituted Benzoates



X–	pH	k_{obsd}, sec^{-1}	k_2, M^{-1} sec^{-1}
$-NHCONH_2$			940
$-NHCONH$	9.90	2.20×10^{-1}	1,740
$\lvert$	8.84	1.62×10^{-2}	
CH_3	8.40	6.14×10^{-3}	
	7.80	1.50×10^{-3}	
$-NCONH_2$	10.50	2.56×10^{-1}	556
$\lvert$	10.00	7.40×10^{-2}	
CH_3	9.50	2.63×10^{-2}	
	9.00	7.90×10^{-3}	
	8.50	2.40×10^{-3}	
$-NHCON(CH_3)_2$	13.27	8.40×10^{-2}	0.263
	12.71	1.85×10^{-2}	
	12.33	7.90×10^{-3}	
	12.00	3.77×10^{-3}	
	11.32	6.60×10^{-4}	
–H	13.71	1.10×10^{-1}	0.125
	13.31	4.20×10^{-2}	
	12.71	8.40×10^{-3}	
	12.31	3.50×10^{-3}	
–H	13.47	4.10×10^{-2}	0.100
In presence of 1.0	13.30	2.70×10^{-2}	
M urea	13.10	1.80×10^{-2}	
	12.69	7.00×10^{-3}	
	12.38	3.38×10^{-3}	
Methyl 4-ureido-	13.71	3.80×10^{-2}	0.025
benzoate	13.31	1.50×10^{-2}	
	12.74	3.78×10^{-3}	
	12.31	1.50×10^{-3}	

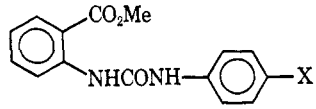
methyl esters of 2-ureidobenzoates which carry one or more methyl groups on the ureido nitrogen adjacent to the aryl ring (N) or the terminal nitrogen (N'). The relative rates are as follows

	Unsubst	N–CH_3	N'–CH_3	N',N'–$(CH_3)_2$
k_{rel}	3580	2110	6600	1

Except in the case of methyl 2-(*N'*,*N'*-dimethylureido)-benzoate, the product obtained was the corresponding quinoxazoline. No participation by the ureido group was evident with the dimethyl compound, 2-(*N'*,*N'*-dimethylureido)benzoic acid being the product obtained.

(b) *N'*-Aryl. The effect of the variation of the nature of substitution on the *N'*-terminal nitrogen of the ureido group was also investigated (Table II). The *N'*-phenyl derivative ($k_2 = 1.00 \times 10^5$ M^{-1} sec^{-1}) reacts *ca.* 100 times more readily than the unsubstituted analog (k_2 = 940 M^{-1} sec^{-1}). Variation of the para substituent in this ring, however, does not bring about a dramatic change in the rate constants. Thus a plot

Table II. Observed and Second-Order Rate Constants for Cyclization of *N*-(2-Carboxymethyl)-*N'*-arylureas

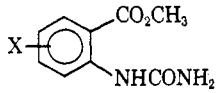


X	pH	k_{obsd}, sec^{-1}	10^5k_2, M^{-1} sec^{-1}
H	8.00	1.34×10^{-1}	1.00
	7.60	5.06×10^{-2}	
	7.00	1.53×10^{-2}	
	6.20	2.17×10^{-3}	
Cl	7.70	1.39×10^{-1}	2.46
	7.40	6.60×10^{-2}	
	7.20	4.30×10^{-2}	
	6.70	1.48×10^{-2}	
	6.40	7.20×10^{-3}	
	5.60	3.50×10^{-3}	
CH_3	8.05	9.60×10^{-2}	0.74
	7.55	4.20×10^{-2}	
	7.05	1.22×10^{-2}	
	6.50	3.25×10^{-3}	
	6.20	1.60×10^{-3}	
NO_2	7.00	7.30×10^{-2}	5.25
	6.00	7.00×10^{-3}	
	5.00	8.08×10^{-4}	

(Figure 2) of log k_2 (for *p*-chlorophenyl, *p*-nitrophenyl, *p*-tolyl, and phenyl derivatives) *vs.* the σ constants of McDaniel and Brown,[21] gives a sensitivity constant, $\rho = \pm 0.87$.

(c) Aryl. The acceleration of the cyclization of methyl 2-ureidobenzoate caused by the introduction of two electron-withdrawing substituents (4-NO_2 and 5-Cl) into the aryl ring was also studied; the resultant rate constants are listed in Table III. Although sufficient data are not available to permit the detailed separation of the various substituent effects that are operative, it is clear from Table III that even NO_2 substitution does not greatly change the rate. Analysis of this data using Hammett-type plots either with σ^- or σ_p for NO_2 and σ_m for Cl or with σ_p for Cl and σ_m for NO_2 (assuming that either the effect on the ester linkage or the ureido group was dominant) gave indifferent plots, *i.e.*, neither of the treatments was demonstrably superior. In any event, the maximal calculable ρ value is *ca.* +1.0.

Table III. Observed Pseudo-First-Order and Second-Order (k_2) Rate Constants for Cyclization of Methyl 2-Ureido-Substituted Benzoates



Substituent X	pH	k_{obsd}, sec^{-1}	k_2, M^{-1} sec^{-1}
H	*a*	*a*	940
5-Cl	9.98	2.40×10^{-1}	1550
	9.93	2.00×10^{-1}	
	8.91	1.80×10^{-2}	
	7.92	1.88×10^{-3}	
4-NO_2	8.98	8.50×10^{-2}	6170
	8.00	9.20×10^{-3}	
	7.50	2.55×10^{-3}	

[a] See Figure 1.

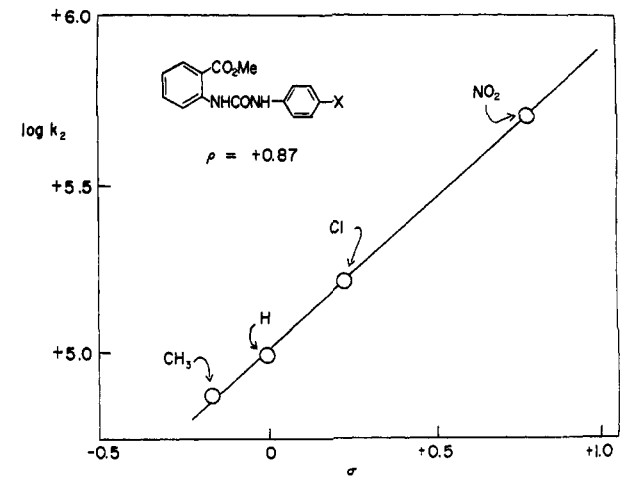
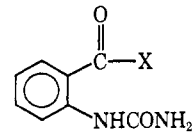


Figure 2. Hammett plot for rates of cyclization of the indicated compounds.

(d) Leaving Group. In Table IV are listed data obtained by the variation of the leaving group when the intramolecular nucleophile is the unsubstituted ureido group. Further data, relating to substituted phenoxides as leaving groups, are also presented in Figure 3. With poor apparent leaving groups, *e.g.*, CH_3O^-, NH_2^-, O^{2-}, only the anionic rates (k_2) were observed and in all cases the product was the benzoxazoline.

Table IV. Observed and Second-Order Rate Constants (k_2) for the 2-Ureidobenzoyl Derivatives



-X	pH	k_{obsd}, sec^{-1}	k_2, M^{-1} sec^{-1}
$-OCH_3$			940
$-OCH_2CF_3$	9.35	2.10×10^{-1}	6,760
	8.86	6.65×10^{-2}	
	7.87	7.20×10^{-3}	
	7.32	2.04×10^{-3}	
	6.86	6.75×10^{-4}	
$-SCH_3$	10.00	1.35×10^{-1}	996
	9.50	4.25×10^{-2}	
	9.00	1.24×10^{-2}	
	8.50	4.22×10^{-3}	
	8.00	1.39×10^{-3}	
$-NH_2$	13.01	2.09×10^{-1}	1.26
	12.09	2.19×10^{-2}	
	11.08	2.15×10^{-3}	
	10.05	2.17×10^{-4}	
$-NHC_6H_5$	12.37	1.53×10^{-1}	2.70
	11.93	4.84×10^{-2}	
	10.94	5.10×10^{-3}	
	10.40	1.58×10^{-3}	
$-N(CH_3)_2$	13.03	2.98×10^{-1}	1.48
	12.02	3.42×10^{-2}	
	10.97	2.95×10^{-3}	
	10.59	1.28×10^{-3}	
$-O^-$	13.70	4.10×10^{-4}	0.00056
	13.01	8.50×10^{-5}	
	12.61	3.40×10^{-5}	
	12.02	1.50×10^{-5}	
	11.34	1.70×10^{-6}	

(21) D. H. McDaniel and H. C. Brown, *J. Org. Chem.*, 23, 420 (1958).

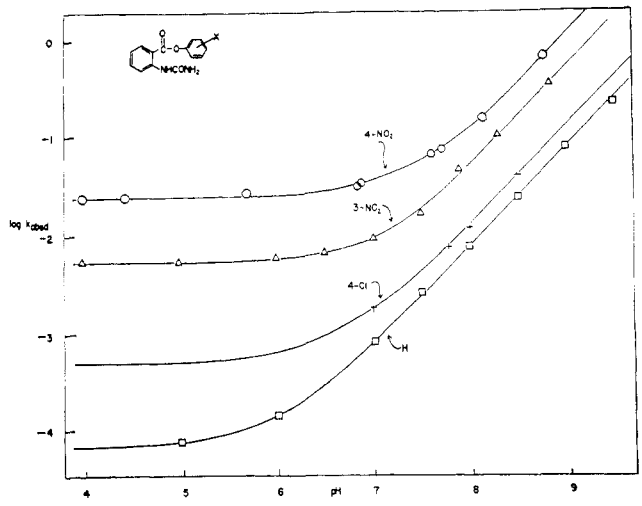


Figure 3. pH–log k_{obsd} profile for cyclization of substituted phenyl *o*-ureidobenzoates (30°, μ = 1.0, H_2O; rate constants in sec^{-1}).

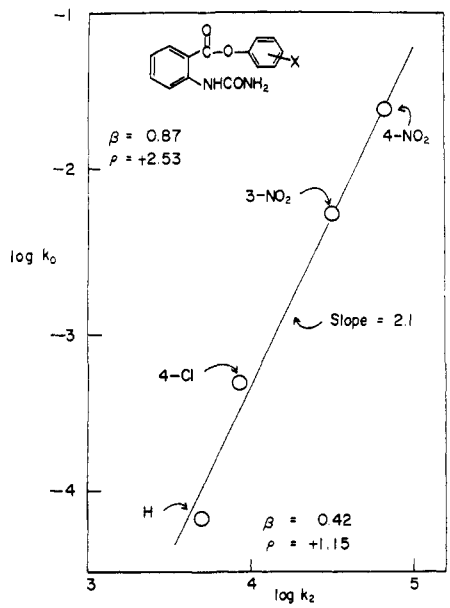
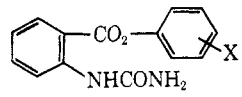


Figure 4. Plots of the logs of the spontaneous (k_0, sec^{-1}) *vs.* the second-order hydroxide ion catalyzed (k_2, l. mol^{-1} sec^{-1}) rate constants for cyclization of substituted phenyl *o*-ureidobenzoates.

When the leaving group was improved ($pK_a < 10$) the intervention of a term (k_0) independent of pH was observed. The importance of the k_0 term increased rapidly with decreasing pK_a of the leaving group, *e.g.*, *p*-nitrophenyl 2-ureidobenzoate has a $t_{1/2}$ for spontaneous cyclization of *ca.* 30 sec. The log k_{obsd} *vs.* pH curves in Figure 3 were analyzed by means of eq 2; the k_0 and k_2 values which best fit this data are presented in Table V. That the spontaneous (k_0) cyclizations were far more sensitive to the nature of the leaving group than the corresponding anionic (k_2) rates is demonstrated in Figure 4 where a plot of log k_0 *vs.* log k_2 yields a slope of 2.1. This may also be expressed in terms of the different Brønsted β's calculated for both processes. Plots of log k_2 and of log k_0 *vs.* the pK_a of the leaving group gave slopes (or β's) of 0.42 and 0.87, respectively. Similarly, plots of log k_2 and log k_0 *vs.* σ (using the values of McDaniel and Brown[21] for *m*-NO_2 and *p*-Cl and +1.0 for *p*-NO_2; previously this value, intermediate between σ and σ^- for NO_2 has been found to be applicable to ester hydrolyses and aminolyses[22]) gave ρ's of +1.15 for anionic attack and +2.53 for the spontaneous process.

Table V. Second-Order Rate Constants for HO^--Catalyzed (k_2) and First-Order Rate Constants (k_0) for Spontaneous Cyclization of Aryl 2-Ureidobenzoates



Substituent X	k_0, sec^{-1}	k_2, M^{-1} sec^{-1}
H	0.000070	5,000
p-Cl	0.00050	8,700
m-NO_2	0.00535	32,400
p-NO_2	0.024	69,200

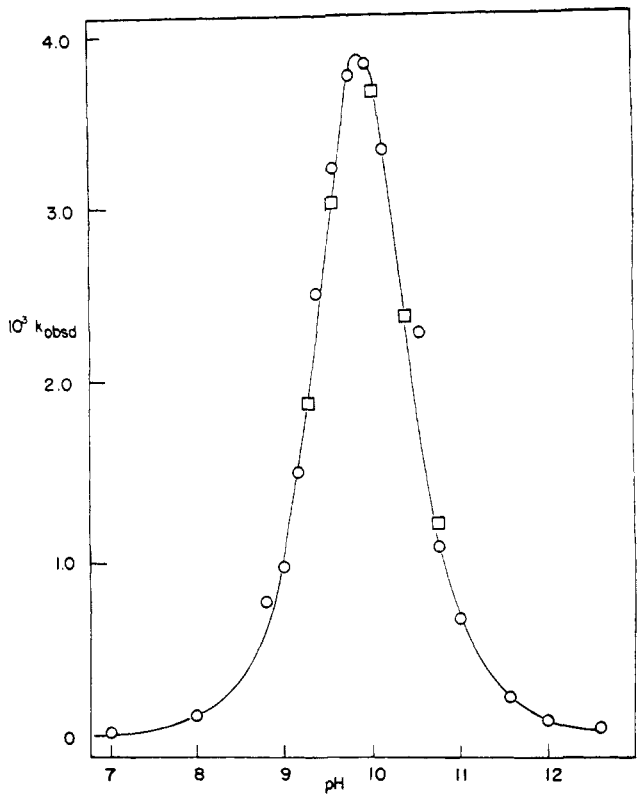


Figure 5. Plot employed for identification of product **4** from cyclization of *m*-nitrophenyl *o*-ureidobenzoate. The circles represent the pH-dependent pseudo-first-order rate constant (k_{obsd}, sec^{-1}) for hydrolysis of authentic **4** while the squares represent the pH-dependent pseudo-first-order rate constants for hydrolysis of the reaction product (30°, H_2O, μ = 1.0).

Accompanying the change in mechanism as the leaving group was improved was a change in product. In the pH-independent region, spontaneous cyclization resulted from oxygen attack by the neutral ureido group with the formation of 2-amino-4,5-benzo-6-oxo-1,3-oxazine. This was identified both spectrally and by a kinetic method. With, *e.g.*, *m*-nitrophenyl 2-ureidobenzoate as substrate the cyclization was allowed to go to completion at pH 5.0. At this pH (see Figure 3), the contribution of the anionic term is negligible. The pH was then rapidly increased to some value in the

(22) T. C. Bruice, A. Donzel, R. W. Huffman, and A. R. Butler, *J. Amer. Chem. Soc.*, **89**, 2106 (1967).

region 9–11 and held constant by means of the pH-stat while the decrease in optical density at 265 nm was recorded as a function of time. The pseudo-first-order rate constants calculated from these data were compared with those obtained for the hydrolysis of authentic 2-amino-4,5-benzo-6-oxo-1,3-oxazine. The oxazine has a characteristic pH–rate profile,[10] particularly in the alkaline region where a maximum at pH 9.91 is observed. In Figure 5 are plotted the observed rate constants for the hydrolysis of the product formed on spontaneous cyclization of *m*-nitrophenyl 2-ureidobenzoate together with data for the authentic oxazine. That the observed constants fit the unique oxazine profile establishes the identity of both materials. In addition, the total decrease in OD at 265 nm could be used, by comparison with a standard, as a measure of the amount of oxazine formed in the preliminary cyclization.

With the poorest leaving groups in which a k_0 term was observed, the rate of ring opening of the oxazine formed (to give 2-ureidobenzoic acid) was of the same order of magnitude as the initial cyclization. Thus at pH = 5.0, for cyclization of phenyl 2-ureidobenzoate, $k_{obsd} = 7.7 \times 10^{-5}$ sec^{-1} ($k_0 = 7.0 \times 10^{-5}$ sec^{-1}), while at the same pH hydrolysis of the oxazine has a rate constant of 2.50×10^{-5} sec^{-1}. Repetitive scans through the ultraviolet at this pH gave, after 6 hr, a spectrum close to that of the oxazine (see Figure 6) but distorted due to the presence of phenol. After a further 72 hr, hydrolysis of the oxazine was essentially complete and the spectrum was that of 2-ureidobenzoic acid (Figure 6).

At higher pH's, where the cyclization occurred entirely through the anion (k_0 was negligible), a small amount of ureido oxygen attack was also observed when a good leaving group was employed. The spectral method used to determine the relative amounts of O and N attack by the ureido anion is detailed in the experimental section. The results obtained are listed in Table VI. In one case O-anion attack is the dominant

Table VI. Relative Amounts of 2-Amino-4,5-benzo-6-oxo-1,3-oxazine (Oxyanion Attack) and 2,4-(1*H*,3*H*)-Quinazolidinedione (Nitrogen Anion Attack) Formed on Cyclization of Aryl 2-Ureidobenzoates

Leaving group	% oxazine	% quinazoline
CH_3O^-	0	100
$C_6H_5O^-$	8	81
p-$ClC_6H_4O^-$	12	89
m-$NO_2C_6H_4O^-$	44	52
p-$NO_2C_6H_4O^-$	58	46

route, becoming more important as the pK_a of the leaving group is decreased. With pK_a > 13, no O-anion attack was detected.

The Brønsted β value of 0.42 quoted earlier for hydroxide-catalyzed cyclizations with substituted phenoxides as leaving groups was calculated from data in Table V, but the k_2 values employed are composite, representing differing amounts of O^- and N^- attack. Combination, however, with the data in Table VI permits the calculation of individual β's of 0.28 for leaving group variation when ureido-N^- is the attacking group and of 0.74 for ureido-O^- attack.

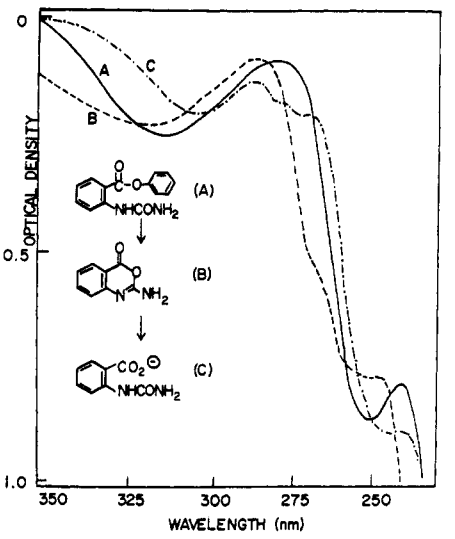


Figure 6. Spectrophotometric time course for the indicated A → B → C reaction (pH 5, 30°, H_2O, μ = 1.0). Spectra of A at t_0, B after 6 hr, and C after t_∞.

Amide *vs.* Ureido Neighboring Groups. The cyclization of 2-ureidobenzamide to 2,4-(1*H*,3*H*)-quinazolinedione can conceivably occur by participation of the ureido or of the amide group. In both cases the leaving group is the same. Two methods were used to determine the relative amount of quinazoline formed by each pathway.

(a) Kinetic. The rate of hydroxide-catalyzed hydrolysis of 2-ureido-*N*,*N*-dimethylbenzamide is given in Table IV as 1.48 M^{-1} sec^{-1}. This is close to the value of 1.26 M^{-1} sec^{-1} for 2-ureidobenzamide. Since the dimethyl substituent effectively blocks amide (anion) attack, ureido participation would appear the dominant pathway. This was confirmed by measuring the rate of cyclization of 2-(*N'*,*N'*-dimethylureido)benzamide; the k_2 for this compound is 5.7×10^{-3} M^{-1} sec^{-1}. Thus, ureido participation appears to be favored by a factor of 1.48/0.0057 = 260.

(b) Product. The cyclization of 2-ureidobenzamide to 2,4-(1*H*,3*H*)-quinazolinedione was also studied under various conditions using a labeling technique. The terminal nitrogen of the ureido group was enriched with ^{15}N and the per cent ^{15}N at the N-3 position of the quinazoline formed was estimated by mass spectrometry. The conditions used for cyclization and the results obtained are summarized in Table VII.

Table VII. Percentage of ^{15}N Retained on Cyclization of *N*-(*o*-Benzamido)-*N'*-[^{15}N]-urea to 3-[^{15}N]-2,4-(1*H*,3*H*)-Quinazolinedione under Various Conditions

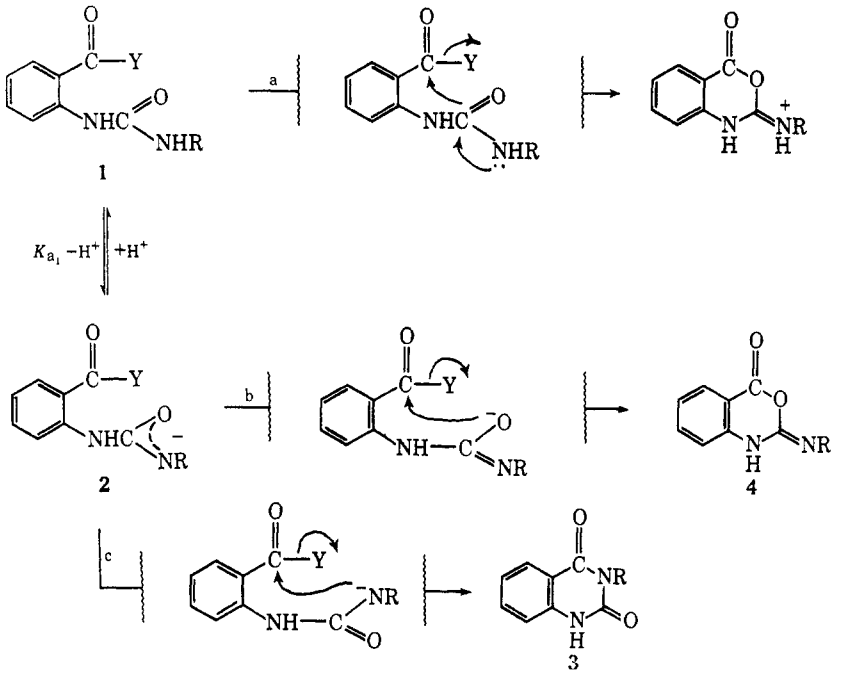
Conditions[a]	% ^{15}N in quinazoline[b]
HO^-	91
H^+	101
Pyridine, reflux	0
Pyrolysis	0
H_2O	0
Electron impact	29

[a] See Experimental Section for details. [b] 100% represents ejection of amide $-NH_2$, 0%, ejection of ureido $-NH_2$ group.

Discussion

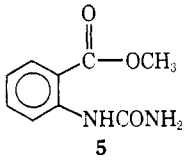
Mechanisms of Ureido Group Participation. This study establishes that the mode of anchimeric partici-

Scheme I

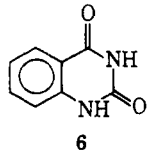


pation by the ureido function in compounds of structure **1** is dependent upon the nature of the leaving group, three mechanisms having been established (Scheme I). In what follows, a discussion of the importance of paths a, b, and c (Scheme I) and the sensitivity of each to electronic effects will be provided.

Path c, the cyclization of methyl 2-ureidobenzoate (**5**) to the quinazoline (**6**), has been most extensively studied. The observed rates of conversion of **5** to **6** were determined over an extended range of pH (Figure 1) and found to be dependent upon the first power of the hydroxide ion concentration. This is consistent



5

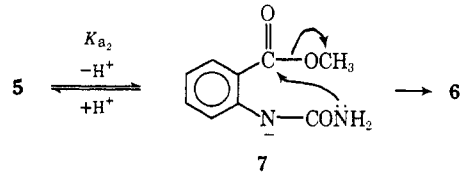


6

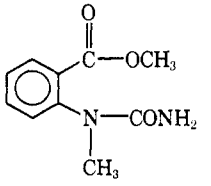
with a kinetic scheme in which the active nucleophile is the ureido anion **2**, which attacks the acyl center through nitrogen. Since the plot of log k_{obsd} *vs.* pH is linear with a slope of +1.0, and does not tend to plateau either at high or low pH, it is apparent that (a) pK_{a_1} is greater than 14 and (b) intramolecular participation by the neutral ureido group is not important when the leaving group is methoxide. General bases, such as glycine, do not accelerate the cyclization (see Table VIII). Therefore specific base catalysis (as in path c of Scheme I) is the preferred mode of reaction.

Anion formation by removal of the proton from the nitrogen adjacent to the ring might be expected to be a more facile process (*i.e.*, $pK_{a_1} > pK_{a_2}$), and it was considered that this anion, **7**, could be the reactive species, the increased electron density on this nitrogen facilitating attack by the terminal ureido nitrogen (Scheme II). The rate of cyclization of the *N*-methyl derivative **8**,

Scheme II



however, is similar to that observed for the unsubstituted derivative **5** (the k_2 constant for **5** is actually 1.7-fold greater, see Table I). Since **8** cannot form the anion **7**, it is unlikely that Scheme II represents the major reaction pathway for **5**. Neither does monomethylation of the terminal nitrogen cause a great



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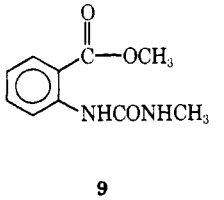

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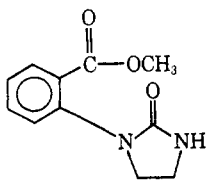

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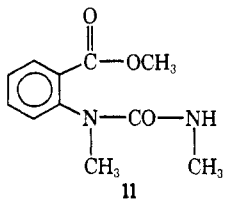

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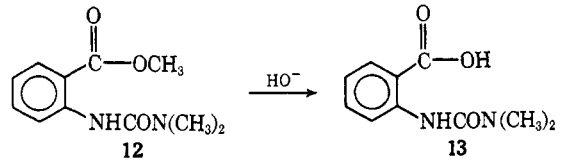
change in the rate of cyclization. Thus, **9** reacts *ca.* 1.8-fold more rapidly than **5** (Table I).

Table VIII. Observed Pseudo-First-Order Rate Constants for Cyclization of Methyl 2-Ureidobenzoate in Glycine Buffers (μ = 1.0, 30°)

[Glycine]$_T$[a]	pH	$10^2 k_{obsd}$, sec^{-1}
1.0	9.70	5.00
0.8	9.69	5.05
0.6	9.69	5.20
0.4	9.69	5.10
0.2	9.69	5.20
		av = 5.11

[a] Total glycine concentration (pK_a = 9.63).

Further support for path c of Scheme I is supplied by the observed behavior of methyl 2-(*N'*,*N'*-dimethylureido)benzoate **12**. Anion formation at the terminal ureido nitrogen is not possible with **12**; the product obtained was the 2-ureidobenzoic acid **13** resulting from hydroxide ion attack at the ester linkage. The observed rate constants also reflect this change in mechanism (Table I). The observed constant for **12** is comparable to that for methyl benzoate under the same con-

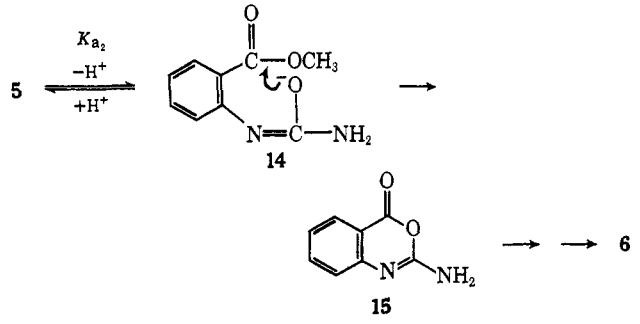


12 **13**

ditions and is 3580-fold less than the cyclization rate of **5**.

The possibility of a stepwise process (Scheme III) involving preliminary displacement of the methoxy group by the oxygen of the ureido anion **14** (or **2**) might also be considered. The hydrolysis of the ini-

Scheme III



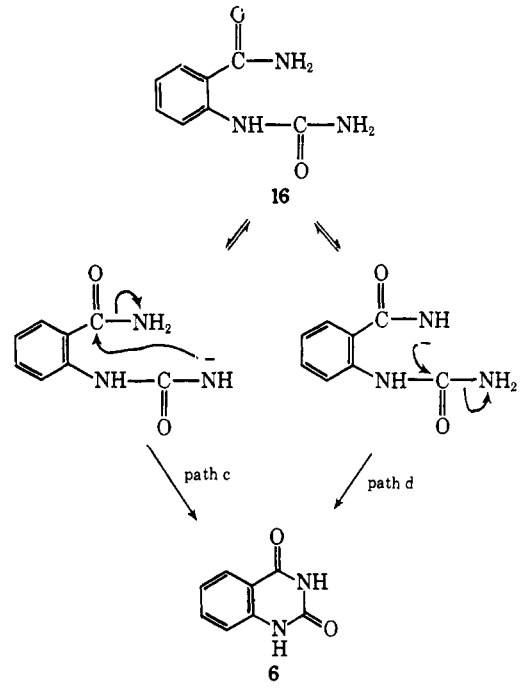
14 **15**

tial product formed, the benzoxazine **15**, has been investigated in detail elsewhere.[10] In alkaline solution, where the formation of **6** is rapid, the hydrolysis of **15** is relatively slow. Therefore if **15** were formed it would accumulate in solution—this was not observed. Moreover the conversion of **15** to **6** does not occur *via* an intramolecular rearrangement; invariably 2-ureidobenzoic acid was formed by the initial hydrolysis of **15** and cyclized relatively slowly to **6**.[10]

Competitive Amide *vs.* Ureido Participation. Several examples are known of hydrolytic reactions which occur by nucleophilic participation of an amide function.[23] Interest in these reactions follows from the predominance of this linkage in proteins and substrate molecules and the possibility that these groups might play an important role in enzymatic processes. The rate increases observed on approximation of the amide group are amongst the largest observed for any group.[8,24,25] It is of interest, therefore, to compare directly the activities of the ureido and amide groups in this respect.

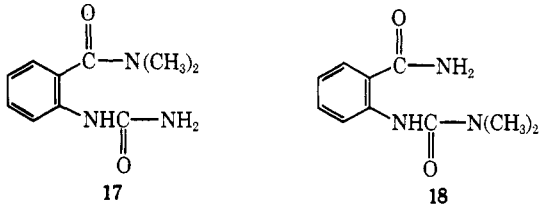
The cyclization of 2-ureidobenzamide **16** to the quinazoline **6** provides a unique opportunity to do this. As shown in Scheme IV, the same leaving group is expelled and the same product is formed for the two reaction pathways. However, by successively blocking amidate anion (in **17**) and ureido anion formation (in **18**), it is

Scheme IV



16 **6**

clearly shown that in Scheme IV, path c is favored. N,N-Dimethylation of the amide resulted in only a small change in the rate of cyclization, *i.e.*, k_2 for **17** is approximately the same as that for **16**, but N',N'-di-



17 **18**

methylation of the terminal ureido nitrogen resulted in a precipitous drop in the rate (the rate of cyclization of **16** is 260 times that observed for **18**). A study of the product formed also agrees with this interpretation. By labeling the terminal nitrogen of the ureido group with ^{15}N it was possible to determine the relative amounts of **6** formed by either pathway in Scheme IV. On cyclization under basic conditions most of the label was retained in **6** (Table VII), indicating that **6** was formed largely by path c.

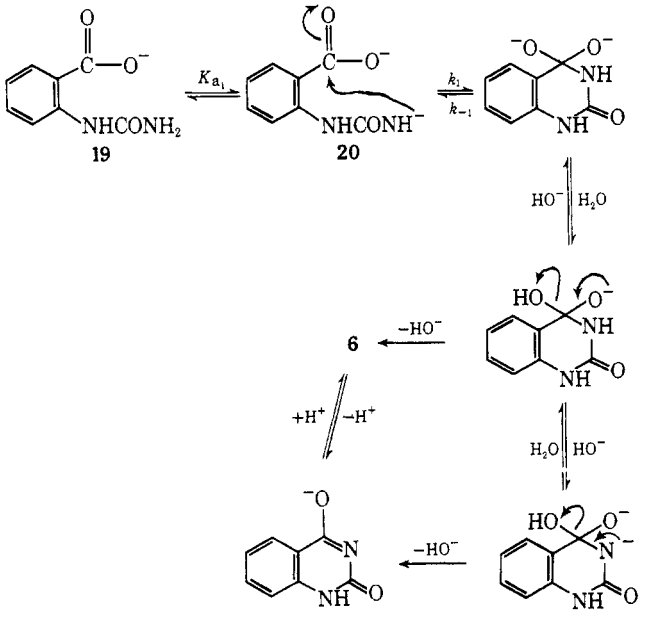
Cyclization of **16** to **6** was also studied under several other conditions (Table VII). Specific acid-catalyzed cyclization (which has a rate constant = 2.5×10^{-4} M^{-1} sec^{-1} at 30°) also occurs *via* ureido group participation. Presumably reaction therefore occurs by nucleophilic attack by the free ureido group on the protonated amide. Why this is so is not readily apparent since phenylurea is more basic (pK_a of the conjugate acid = −0.3 at 25°) than benzamide (pK_a = −2.16 at 25°).[26] Even more surprising is that, in refluxing anhydrous pyridine, cyclization occurs entirely by the other path (d), that is, by amide attack. Although this result is unusual, a similar conclusion could be inferred from previous data for the cyclization of compounds related to **16** but with a single substit-

(23) From a compilation of recent literature; see ref 24.
(24) S. C. K. Su and J. A. Schafer, *J. Org. Chem.*, **34**, 926 (1969); and M. T. Behme and E. H. Cordes, *ibid.*, **29**, 1255 (1964).
(25) J. A. Schafer and H. Morawetz, *ibid.*, **28**, 1899 (1963).
(26) D. D. Perrin, "Dissociation Constants of Organic Bases," Butterworths, London, 1965.

uent on the amide portion.[17] In these substituted compounds different products are formed by routes c and d and the amount formed by each route was estimated. The reversal of the order ureido > amide in pyridine solution is probably not due to some specific effect of the medium since in the absence of solvent pyrolysis of **16** to **6** also goes *via* path d. The same result, but to a lesser extent, was observed when the cyclization by electron impact on labeled **16** was studied in the mass spectrometer. Over two-thirds of **6** was formed by loss of the terminal ureido nitrogen.

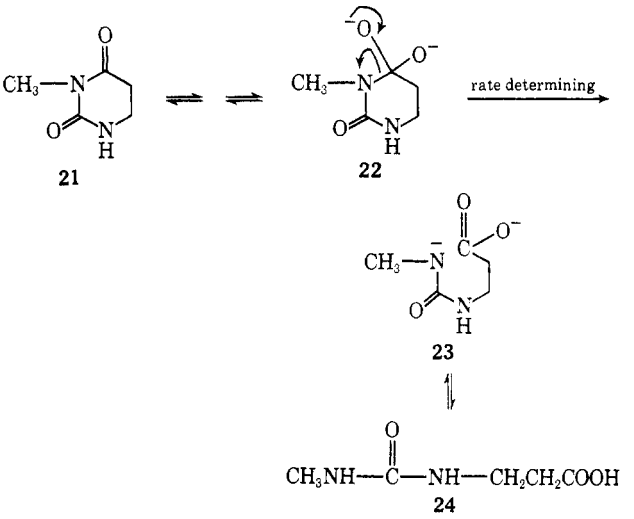
Cyclization of 2-Ureidobenzoic Acid. Of particular interest is the observation that, at high pH, the rate of cyclization of 2-ureidobenzoic acid (**19**) is proportional to the hydroxide ion concentration. This is consistent with the mechanism[27] in Scheme V, where the rate-

Scheme V



determining step is nucleophilic attack by the ureido anion on the carboxylate anion (k_1). Without considering the electrostatic barrier to this process (which, *a priori*, should be very large), such a reaction is not unreasonable. Thus the pK_a of the conjugate acid (HO^-) of the group expelled is[28] *ca.* 25, whereas the ureido anion can displace with relative ease leaving groups as basic as NH_2^- (where the pK_a of the conjugate acid, ammonia, is *ca.* 35.[29]

The kinetics of hydrolysis of 3-methyl-5,6-dihydrouracil (**21**) show a dependence on hydroxide ion concentration which changes from second order to first order as $[HO^-]$ is increased.[30] Several kinetically equivalent mechanisms (which may or may not be general base catalyzed) were considered to explain these results. The most favored involves rate-determining breakdown of a dianionic tetrahedral intermediate **22**, to give initially the species **23**. If this is correct then the reverse reaction, the cyclization of **24** to **21**, should also proceed through a rate-determining attack by ureido anion on



carboxylate anion. The similarity of this to the mechanism proposed in Scheme V is obvious.

To obviate the involvement of ureido anion attack on carboxylate anion, an alternative pathway (Scheme VI) is also considered. Since the cyclization occurs in a pH region well above the dissociation constant of the carboxyl group (pK_{a_2}) and below the dissociation constant of the urea (pK_{a_3}), the 2-ureidobenzoic acid is present almost entirely in the form of **25**. The concentration of the isomer monanion **27** is therefore invariant with pH. The derived expression from this scheme is given in eq 3; the species **27** and **28** were assumed to be in steady-state concentrations. Since $K_{a_2} \gg (a_H + K_{a_3})$, this equation simplifies to eq 4.

$$k_{obsd} = \frac{k_2 K_{a_4} k_1}{(k_{-1} a_H + k_2 K_{a_4})} \frac{K_{a_3}}{K_{a_3} + a_H + K_{a_2}} \quad (3)$$

$$k_{obsd} = \frac{K_{a_4} k_1 k_2}{(k_{-1} a_H + k_2 K_{a_4})} \frac{K_{a_3}}{K_{a_2}} \quad (4)$$

If k_2 is rate determining (*i.e.*, $k_{-1} \gg k_2 K_{a_4}/a_H$), then the expression is reduced to eq 5, which is of the correct

$$k_{obsd} = \frac{k_1 k_2 K_{a_3} K_{a_4}}{k_{-1} K_{a_2} a_H} \quad (5)$$

form to account for the kinetic results. That this is the correct mechanistic interpretation, however, is doubtful for the following reasons.

At very low a_H (*i.e.*, high pH), $k_2 K_{a_4}/a_H$ may become greater than k_{-1}, *i.e.*, k_1 is rate determining. Equation 4 reduces to eq 6, so that the observed rate of cyclization should be *independent* of pH. An estimate of this plateau rate can be made using the cyclization of **5** as a model. For this reaction

$$k_{obsd} = k_2' K_{a_1}/a_H$$

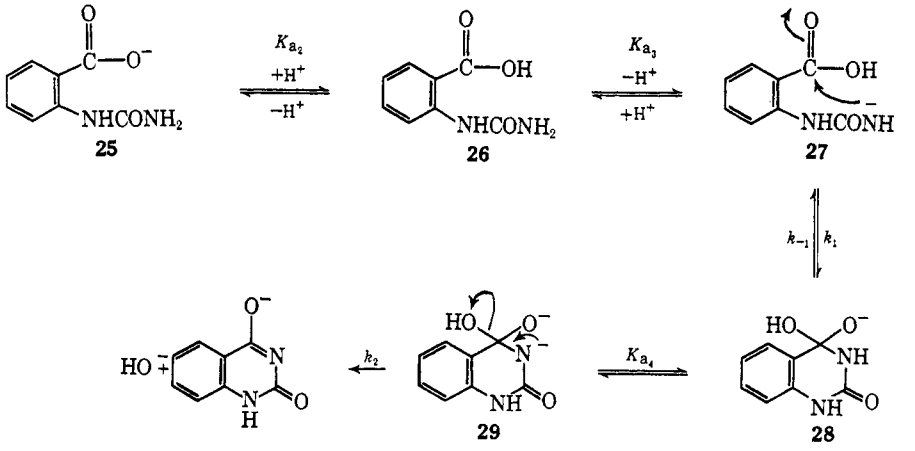
$$k_2' K_{a_1} = k_{obsd} a_H = 1.38 \times 10^{-11}\ M\ sec^{-1}$$

If the reasonable assumptions are made that K_{a_3} (Scheme VI) = K_{a_1} (Scheme I) and that $k_1 = k_2'$ (since the pK_a's of the leaving groups are approximately the same), then the plateau rate may be calculated as 3.2×10^{-8} sec^{-1} using eq 6 and the known[10] K_{a_2} value (4.35×10^{-4}).[31]

$$k_{obsd} = k_1 \frac{K_{a_3}}{K_{a_2}} \quad (6)$$

(27) A. F. Hegarty and T. C. Bruice, *J. Amer. Chem. Soc.*, **91**, 4924 (1969).
(28) R. P. Bell, "The Proton in Chemistry," Methuen, London, 1959, p 92.
(29) See ref 28, p 87.
(30) E. G. Sander, *J. Amer. Chem. Soc.*, **91**, 3629 (1969). See also, I. Blagoeva, B. J. Kurtev, and I. G. Pojarlieff, *J. Chem. Soc. B*, 232 (1970).
(31) Note that a similar calculation was successful in predicting the pH-independent rate of cyclization of 2'-carboxy-4-bromocarbanilide [P. J. Taylor, *J. Chem. Soc. B*, 1554 (1968)].

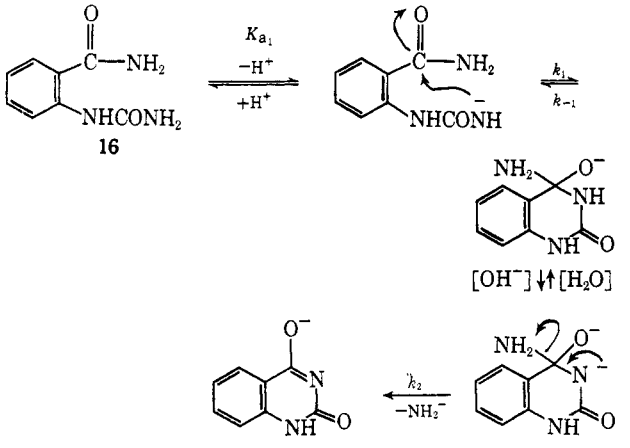
Scheme VI



No such plateau was observed at high pH and, more important, the observed rate constants for cyclization are, at $[HO^-] = 1.0\ M$, $\sim 10^4$-fold greater than that calculated for the maximal plateau value of Scheme V.

A further consideration also casts doubt on Scheme VI. The corresponding cyclization of 2-ureidobenzamide **16** could also conceivably occur by a mechanism similar to that of Scheme VI; this is outlined in Scheme VII. If k_2 is rate determining (as it would have to be in

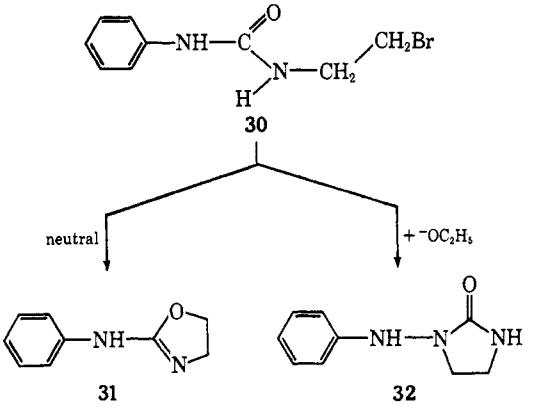
Scheme VII



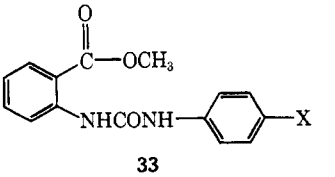
Scheme VI) then $k_{obsd} \propto [HO^-]$,[2] rather than $[HO^-]$, as observed (Table IV). Therefore, in this case k_1 is rate determining. Note that both reactions were studied in approximately the same pH range and the pK_a's of the intermediate which could be formed in both cases should be similar.

Path b becomes of importance when $-Y$ of **1** represents a good leaving group (Table VI). This mode of anchimeric attack becomes evident as the pK_a of HY decreases below 11. As $-Y$ becomes an increasingly better leaving group the percentage of product representing the benzoxazine (**4**) increases rapidly at the expense of the N-anion attack product (**3**). In fact, when Y = 4-$NO_2C_6H_4O$- (pK_a of HY is then 7.14) **4** is the major product. The observed second-order rate constants, k_2, for the cyclization of **1** (R = H) are, therefore, composite when Y = 4-$NO_2C_6H_4O$-, 3-NO_2-C_6H_4O-, 4-ClC_6H_4O-, C_6H_5O-, and CH_3S-, containing rate terms for formation of **4** and **3** (R = H) *via* ureido oxyanion and nitrogen anion attack. From the determined product ratios (Table VI) and the determined rate constants, k_2 (Table V), individual rate constants for paths b and c of Scheme I can be calculated.

Path a, involving participation by the un-ionized ureido group, also becomes apparent as the leaving group ability of Y in **1** (R = H) is improved. The rates of spontaneous cyclization (k_0 in Table V) first become apparent when pK_a of HY is *ca.* 10. The spontaneous reaction results in the formation of the benzoxazine (**4**), rather than the quinazoline (**3**). This is similar to the behavior noted for attack by the neutral ureido group on sp^3 carbon; for the neutral ureido group the nucleophilic center is oxygen (**31** is formed) while cyclization of **30** catalyzed by ethoxide ion results in formation of the N-attack product.[32] Similarly the nucleophilic center of the neutral amide group is generally the carbonyl oxygen, whereas the nucleophilic center of the anion is usually nitrogen.[33]



Sensitivity of Reaction Pathways to Electronic Effects. Dependence of rate on the acidity of the ureido group has been investigated by determining the rates of cyclization of compounds of type **33**. The low sensitivity



(32) S. Winstein and R. Boschan, *J. Amer. Chem. Soc.*, 72, 4669 (1950); F. L. Scott, R. E. Glick, and S. Winstein, *Experientia*, 13, 183 (1957).

(33) T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms," W. A. Benjamin, New York, N. Y., 1966, Chapter 1.

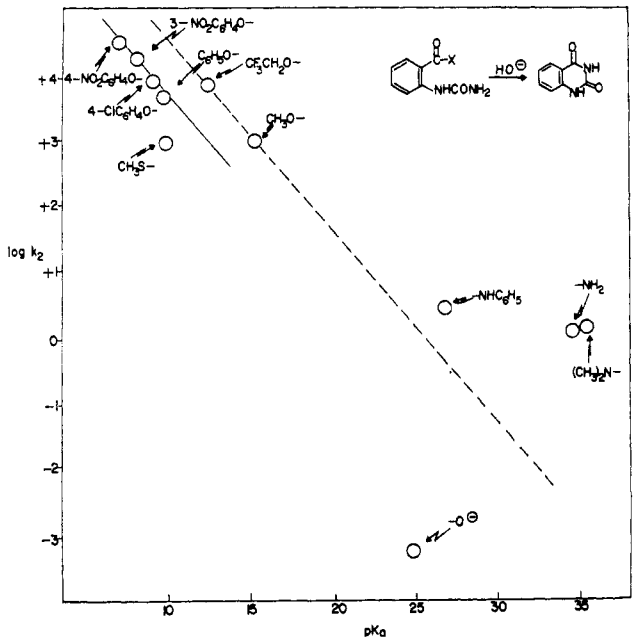


Figure 7. Plot showing dependence of the log of the second-order rate constants for hydroxide ion catalyzed cyclization (l. mol^{-1} sec^{-1}) of indicated compounds upon the pK_a values of the conjugate acid of the leaving groups (-X).

to substituent variation in the N'-aryl ring of **33** reflects the fact that the ρ_{obsd} (+0.87, see Figure 2) is composite. A given substituent change acts on the acidity of the N'-ureido hydrogen (K_{a_i}, see Scheme I) and on the nucleophilicity of the anion formed (k_2') in opposing directions, an increase in acidity being offset by a decrease in the nucleophilicity of the anion formed. Both K_{a_i} and k_2' are contained in k_2 (see eq 2), which was used to determine ρ_{obsd}. An approximation of the effect of substituent variation on K_{a_i}, however, permits an evaluation of the individual reaction constants.

From the empirical relationship (eq 7) between the

$$pK_{aniline} = 1.3pK_{anilinium\ ion} + 21.4 \qquad (7)$$

pK_a's of anilines and of their conjugate acids, proposed by Stewart and Dolman,[34] $\rho_{aniline} = +3.8$ may be calculated (using[35] $\rho_{anilinium\ ion} = +2.85$). Equation 8 may then be derived from eq 2 as follows

$$\log (k_2/k_2^\circ) = \rho_{obsd}\sigma$$

and

$$\log k_2' = \beta pK_a + C$$

$$\log (K_a/K_a^\circ) = \rho_{ureido}\sigma$$

$$\log (k_2'/k_2'^\circ) = -\beta \log (K_a/K_a^\circ) = -\beta\rho_{ureido}\sigma$$

so that

$$\log (k_2/k_2^\circ) = \log (k_2'/k_2'^\circ) + \log (K_a/K_a^\circ)$$

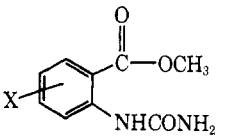
therefore

$$\rho_{obsd} = \rho_{ureido}(1 - \beta) \qquad (8)$$

Making the assumption that aniline and phenylurea acidities respond to the same degree to substituent variation (*i.e.*, $\rho_{ureido} = \rho_{aniline}$) then $\beta = +0.77$. This calculated β value for the dependence of k_2' on the pK_a of the ureido group is almost identical with that obtained (+0.76) from a plot of the second-order rate constants *vs.* pK_a of the alcohol for the reaction of *p*-nitrophenylacetate with alkoxide ions of $pK_a < pK_w$.[36]

The β value for variation of the nature of the leaving group [calculated using compounds of the type **1** (R = H)] is *ca.* 0.3 (Figure 7). Thus the cyclization is more sensitive to the nature of the nucleophilic center than to the nature of the leaving group. This observation (which has also been widely reported in the reactions of strong nucleophiles such as hydroxide ion with less activated esters)[36,37] has been interpreted in terms of nonconcerted bond making and breaking in the transition state: *i.e.*, *N*-acyl bond formation has progressed further in the transition state than acyl oxygen fission.

The effect of substituents in the aryl ring of compounds of the type **34**, again, results in only small changes in reactivity (*e.g.*, a nitrosubstituent para to



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the carbomethoxy increases the rate of cyclization just fivefold). As in the case of **33**, several factors which partly compensate each other are operative. Thus the nitro group would activate the ester to nucleophilic attack by the ureido anion. This follows from studies of the related hydroxide-catalyzed hydrolyses of methyl benzoates which show[38] a positive ρ value (1.93), when the acyl substituent is varied. This is consistent with data for reaction of other nucleophiles such as amines with various acyl-substituted esters like phenyl acetates[39] and phenyl benzoates.[40] The nitro group should also aid anion formation, *i.e.*, increase K_{a_i} in Scheme I; there would also be, as shown above, a corresponding decrease in the nucleophilicity of the anion formed. The changes observed in the rates of cyclization (k_2) of **34** when X was varied were so small that the contribution of these various effects to the overall rate could not be accurately separated. It is clear, however, that for substituents meta or para to the carbomethoxy group, $\rho < 1$.

This result permits the correlation of the data for the variation of the leaving group with more confidence. Since the nucleophilic ureido group is attached to the same aryl ring as is the acyl function, when the nature of the latter is changed then K_{a_i}, k_2', etc., for the ureido function must vary also. The electron-withdrawing power of the acyl function varies in the order $-CO_2R > -CONH_2 > -CO_2^-$ (as judged from the σ_p's for these groups which vary from +0.45 to 0.0). Since the maximum ρ for substituent variation in **34** is 1.0, making the extreme change from $-CO_2R$ to $-CO_2^-$ would result in only a twofold rate change if these substituents were

(34) R. Stewart and D. Dolman, *Can. J. Chem.*, **45**, 925 (1967).
(35) A. I. Biggs and R. A. Robinson, *J. Chem. Soc.*, 388 (1961).
(36) T. C. Bruice, T. H. Fife, J. J. Bruno, and N. E. Brandon, *Biochemistry*, **1**, 7 (1962).
(37) W. P. Jencks and M. Gilchrist, *J. Amer. Chem. Soc.*, **90**, 2622 (1968).
(38) M. L. Bender and R. J. Thomas, *ibid.*, **86**, 4181 (1964); see also J. F. Kirsch, W. Clewell, and A. Simon, *J. Org. Chem.*, **33**, 127 (1968).
(39) T. C. Bruice, A. F. Hegarty, S. M. Felton, A. Donzel, and N. G. Kundu, *J. Amer. Chem. Soc.*, **92**, 1370 (1969).
(40) J. F. Kirsch and A. Kline, *ibid.*, **91**, 1841 (1969).

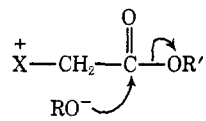
para to the ester function. The observed great changes in k_2 (Figure 7) must represent largely the differing leaving group abilities, electrostatic effect, etc., rather than a change in the nucleophile.

Variation of the Leaving Group. The ureido anion, as a powerful nucleophile, proves to be relatively insensitive to the nature of the group [Y in **1** (R = H)] which is being displaced. Intramolecular displacement of Y by the ureido group could therefore be observed with a variety of deactivated acyl functions: *e.g.*, $-C(=O)NHC_6H_5$, $-C(=O)NH_2$, and even with $-CO_2^-$ (Figure 7).

From the data of Table V the *sensitivities of paths b and c* of Scheme I *to the nature of the leaving group* may be ascertained. As anticipated, O^- attack is more sensitive to the nature of the leaving group ($\beta = 0.74$) than is N^- attack ($\beta = 0.28$). From these data it can be calculated that $<1\%$ of the reaction pathway is *via* O^- when the pK_a of HY is >12, and that N^- attack is similarly negligible if the pK_a of HY is <3. This is consistent with the observed reaction pattern for compounds with poorer leaving groups (which give **3** exclusively) but, for practical reasons, the lower limit (with pK_a's of the leaving groups *ca.* 3) could not be examined.

It is difficult to correlate all of the second-order rate constants, k_2, against the pK_a of the leaving group with any precision on a single plot when large changes in the nature of the leaving group are made (Figure 7). The constants on this plot have been corrected (where required) for reaction of O^- and therefore represent solely N^- attack. The broken line in Figure 7 has been drawn through the points for Y = OCH_3 and OCH_2CF_3 and has a slope of -0.26. This is the same slope obtained in the analogous bimolecular hydroxide catalyzed hydrolysis of meta- and para-substituted phenyl acetates. When Y in **1** (R = H) is substituted phenoxide, the Brønsted line is approximately parallel to the broken line but *ca.* 0.8 log unit below. The one thiol ester studied (**1**, Y = $-CH_3S$, R = H), cyclized at a rate which placed it *ca.* 0.8 log unit below this line. It is probable that if more data were available, the thio esters (and probably the amides) would constitute several groups, each with much the same response to leaving group variation but displaced either above or below the broken line in Figure 7.

The largest deviation (-3.4 log units) from the plot is shown by 2-ureidobenzoate (**1**, Y = O^-, R = H). The cyclization of this compound to **6** is thought to proceed *via* intramolecular attack by the ureido anion on the carboxylate anion. The deviation could be attributed to retardation of the cyclization caused by the electrostatic repulsion to be overcome as the two anions are brought together in the transition state. Bruice and Holmquist[41] have shown by using the reaction of oxyanions with suitable esters containing a charged substituent α to the carbonyl (eq 9) that electrostatic facilitation could be observed in certain instances, *e.g.*, when



(9)

(41) T. C. Bruice and B. Holmquist, *J. Amer. Chem. Soc.*, **89**, 4028 (1967); B. Holmquist and T. C. Bruice, *ibid.*, **91**, 2982, 2985 (1969).

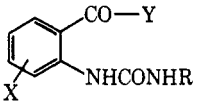
RO^- was a relatively poor nucleophile. In these cases, it was rationalized, the bond between the oxyanion and the acyl group was short enough in the transition state so that the energy barrier was reduced by the propinquity of the oppositely charged groups. With strong nucleophiles such as HO^- and $CF_3CH_2O^-$, the charged esters showed no deviations from linear free-energy plots, presumably because in these cases the transition state was reached early on the reaction coordinate, minimizing charge–charge interaction. The cyclization of 2-ureidobenzoic acid should be in the latter category since the ureido anion is a relatively strong nucleophile but countering this is the fact that the charged center is in this case also the electrophilic center (rather than being α to it as in 9).

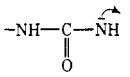
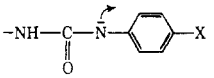
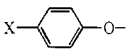
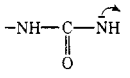
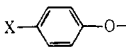
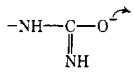
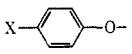
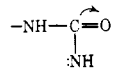
The rates of spontaneous cyclization (k_0 in Table V) are far more sensitive than k_2 to the nature of the leaving group. The fact that the rate of anionic cyclization changes by about the same amount when Y = $-OCH_3$ and Y = $-NH_2$ (where ΔpK_a between the leaving groups is *ca.* 20) as the rates of neutral cyclization do when Y = 4-$NO_2C_6H_4O$ and Y = C_6H_5O ($\Delta pK_a \sim 3$) emphasizes this point. This is also shown by the larger Brønsted β (or Hammett ρ) value obtained (when the leaving group is varied) for k_0 than for k_2 (see Figure 4). This behavior also finds analogy in the intermolecular reactions of esters. With strong oxyanion nucleophiles such as alkoxide or hydroxide ion, β for leaving group variation is usually small (usually *ca.* 0.3),[42] whereas neutral nucleophiles, such as the amines, are far more sensitive to the nature of the leaving group ($\beta \sim 0.8$).[43,44] A summary of the sensitivity of the various paths of Scheme I to electronic effects is provided in Table IX.

The Relative Efficiency of the Ureido Group as Intra- and Intermolecular Nucleophile. In an attempt to calculate the rate acceleration caused by the approximation of the ureido group to the ester in **5**, intermolecular models of the reaction were sought. In Table I are listed the second-order rate constants for hydroxide ion catalyzed hydrolysis of methyl benzoate in aqueous solution (30°, $\mu = 1.0$) and in the presence of 1.0 *M* urea. Rather than observing a rate increase (which might be attributable to urea or ureido anion attack on the ester linkage), a small *decrease* (20%) in the rate was noted in the presence of urea (Table I). Using the standard technique of assuming that *ca.* 5% of the reaction could have occurred by bimolecular attack of the urea anion without detection, a rate acceleration of 1.5×10^5-fold due to approximation may be calculated. Evidence that this rate acceleration is not merely caused by the electronic effect of the ureido group ortho to the ester is provided by comparison with the rates of hydrolysis of methyl 4-ureidobenzoate and methyl 2-(*N'*,*N'*-dimethylureido)benzoate (Table I). The substituent ureido groups in both of these compounds and in **5** should have much the same effect in activating the ester group toward hydroxide ion but only **5** shows a dramatic rate acceleration (relative to, say, the hydrolysis of methyl benzoate).

(42) T. C. Bruice and M. F. Mayahi, *ibid.*, **82**, 3067 (1960).
(43) T. C. Bruice and S. J. Benkovic, *ibid.*, **85**, 1 (1963); **86**, 418 (1964).
(44) L. doAmaral, K. Koehler, D. Bartenbuch, T. Pletcher, and E. H. Cordes, *ibid.*, **89**, 3539 (1967).

Table IX. Summary of the Observed Substituent Effects in the Cyclization of



Y	Nucleophile	X	Sensitivity parameters[a]
CH_3O-		−H, 5-Cl, 4-NO_2	$\rho \leq 1.0$ for k_2[HO^-][SH]
CH_3O-		H	$\rho_{obsd} = +0.87$ for k_2[HO^-][SH] $\beta = +0.77$ for k_2'[S^-]
		H	Log $k_2 = -0.28pK_{phenol} + 6.6$ for k_2[HO^-][SH]
		H	Log $k_2 = -0.74pK_{phenol} + 10.0$ for k_2[HO^-][SH]
		H	Log $k_0 = -0.87pK_{phenol} + 4.74$ for k_0[SH]

[a] [SH] = concentration of un-ionized substrate; [S^-] = concentration of ionized substrate.

The huge rate accelerations noted on conversion of bimolecular attack by neutral urea or urea anion on acyl functions to the corresponding intramolecular analogs is reminiscent of similar rate ratios observed in the comparison of inter- to intramolecular attack of the carboxyl anion.[45] The present study emphasizes again the great importance that approximation of reactant groups may have in the determination of the facility of enzymatic reactions.[46]

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

(45) T. C. Bruice and A. Turner, *J. Amer. Chem. Soc.*, **92**, 3422 (1970).

(46) Based partially on the present study an alternate mechanism for biotin enzyme mediated carboxylation reaction has been proposed.[9]