Acyl Transfer Reactions from and to the Ureido Functional Group. IV. Neighboring Carboxyl Group General Acid Catalysis in the Hydrolysis of an *O*-Acylisourea (2-Amino-8-carboxy-4-oxo-3,1,4-benzoxazine)

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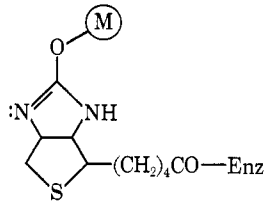
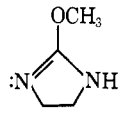
Abstract: 2-Amino-4-oxo-3,1,4-benzoxazine (**5**) has previously been studied as a plausible model for the CO_2–biotin complex of biotin carboxylases. The present paper examines the effect of carboxyl substitution on the reactivity of this model system toward nucleophiles. The compounds used were 2-amino-8-carboxy-4-oxo-3,1,4-benzoxazine and the 6- and 5-carboxyl isomers (**11**, **12**, and **13**, respectively). Kinetic analysis of the hydrolysis rates of these compounds indicates that although a 5-carboxyl group (adjacent to the reactive carboxyl site of the benzoxazine) does not promote hydrolysis, as judged by comparison with the 6-carboxy isomer where participation is impossible, an 8-carboxyl group (adjacent to the benzoxazine nitrogen atom, *i.e.*, the leaving group) markedly does so. The reactivity in the latter case, thought to be that of the species **11b**, is proposed to arise from intramolecular general acid catalysis. The hydrolytic reactivity of **11b** is comparable to that of **5a** or **11a** where the benzoxazine ring nitrogen atom is fully protonated. This catalytic efficiency is also seen in the morpholinolysis of **11**, where **11b** is only a few times less reactive than **5a** and some 10^5 times more reactive than **5b**.

The particular means by which the imidazolidone ureido group of biotin participates in carboxylation reactions is probably not yet well understood.[3] We have recently made two suggestions to explain the transfer of "activated CO_2" to and from the imidazolidone portion of enzyme-bound biotin. Because of the extreme lack of reactivity of the –NH group,[4] it has been suggested that the tertiary nitrogen atom of the imidazoline species (**1**)[5]



1, (M) = metal ion

should be considered as the nucleophilic center of biotin. The nucleophilicity of 2-methoxy-2-imidazoline (**2**) has been estimated to be *ca.* 10^{10} greater than that of imidazolidone (**3**) toward the phenyl ester carbonyl



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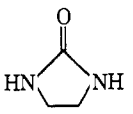

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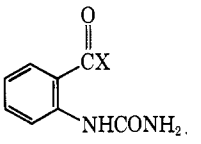
group.[5] The carbonyl oxygen of the ureido function of biotin should also be given consideration as the nucleophilic center of biotin. This suggestion[3] is based on the determined mechanisms of intramolecular nucleophilic attack of the ureido functional group in compounds of general structure **4**. It can be shown that



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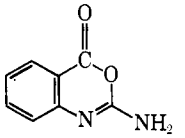
when –X represents a good leaving group (as would be anticipated to be present in "active CO_2"), oxygen attack predominates to yield the oxazine **5**; for the case of poor leaving groups, nitrogen attack provides the quinazoline (**6**).[6] Formation of **5** may occur through



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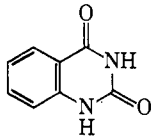

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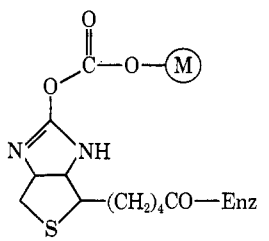
either the ureido anion or the neutral species, while formation of **6** occurs only *via* the ureido anion. These results suggested to us[3] that a structure such as **7** may best describe the active site of CO_2–biotin carboxylases.



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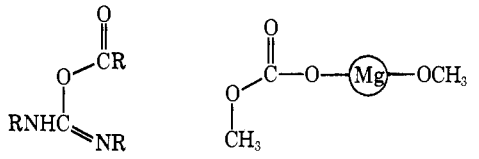
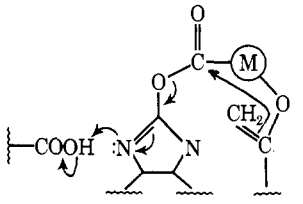
(2) To whom inquiries should be addressed.
(3) T. C. Bruice and A. F. Hegarty, *Proc. Nat. Acad. Sci. U. S.*, **65**, 805 (1970).
(4) (a) M. Caplow, *J. Amer. Chem. Soc.*, **87**, 5774 (1965); (b) M. Caplow and M. Yager, *ibid.*, **89**, 4513 (1967); (c) M. Caplow, *ibid.*, **90**, 6795 (1968).
(5) A. F. Hegarty, T. C. Bruice, and S. J. Benkovic, *Chem. Commun.*, 1173 (1969).
(6) Part III: A. F. Hegarty and T. C. Bruice, *J. Amer. Chem. Soc.*, **92**, 6575 (1970).

Structure **7** is mechanistically pleasing since it embodies the acyl transfer characteristics of an isoimide (as found in the addition product[7] of a carboxylic acid to a carbodiimide (**8**)) and the structural features of the Stiles–Finkbeiner reagent (**9**)[8] for enol carboxylation.



8 **9**

Studies of the chemistry of *syn*-isoimides are difficult since they can rarely be isolated due to rapid O → N acyl migration.[9] Acyl migration is prohibited in 2-amino-4-oxo-3,1,4-benzoxazine (**5**) allowing investigation of the susceptibility of the ester carbonyl group to nucleophilic attack. Studies of the kinetics of the facile hydrolysis and aminolysis of **5** have recently appeared.[10,11] Of the many features of these reactions, one of particular note is the great susceptibility of the acyl carbonyl group to attack when the ring nitrogen of **5** is protonated. This observation suggests that protonation of **7** would increase its group transfer potential (*i.e.*, **10**). The present investigation deals with the



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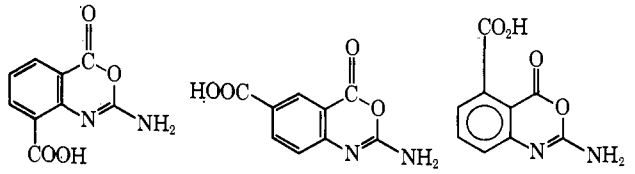
kinetics of hydrolysis and aminolysis of 2-amino-8-carboxy-4-oxo-3,1,4-benzoxazine (**11**) and 2-amino-6-carboxy-4-oxo-3,1,4-benzoxazine (**12**), the former being offered as a model for **10**. The hydrolysis of 2-amino-



11 **12** **13**

5-carboxy-4-oxo-3,1,4-benzoxazine (**13**) has also been examined in order to assess the possibility of carboxyl catalysis at the reactive carbonyl center.

Experimental Section

Materials. 2-Amino-8-carboxy-4-oxo-3,1,4-benzoxazine (11). 2-Aminoisophthalic acid[12] (6.3 g, 0.035 mol) dissolved in 10 ml of water containing 2.8 g (0.07 mol) of sodium hydroxide was added with stirring over 5 min to 6.3 g (0.06 mol) of cyanogen bromide in 15 ml of water. The white precipitate formed was removed by filtration and washed sparingly with water. The free acid was obtained by dissolving the salt in water, rapidly adding acid to pH *ca.* 4, and filtering. Repeated recrystallization of the filtered precipitate from dioxane yielded a white solid which yellowed at 260° and finally melted in the range 320–330°. The infrared spectrum shows strong absorptions at 1600, 1650, 1710, and 1780 cm^{-1}.

Analysis figures for this compound (dried *in vacuo* at room temperature) suggest a 2:1 benzoxazine–dioxane adduct.

Anal. Calcd for $2C_9H_6N_2O_4 \cdot C_4H_8O_2$: C, 52.80; H, 4.02; N, 11.19. Found: C, 52.89; H, 4.22; N, 11.03.

A pmr spectrum (taken in trifluoroacetic acid) confirmed the presence of dioxane (singlet at τ 6.0). An analysis was also obtained on a sample dried *in vacuo* overnight at 100°.

Anal. Calcd for $C_9H_6N_2O_4$: C, 52.43; H, 2.93; N, 13.59. Found: C, 52.50; H, 3.27; N, 13.55.

2-Amino-6-carboxy-4-oxo-3,1,4-benzoxazine (12). This compound was prepared from 4-aminoisophthalic acid[13] in the manner described above. After recrystallization from dioxane the sample turned brown but did not melt below 360°. Analysis (dried *in vacuo* at room temperature) fits a dioxane adduct as for **11**.

Anal. Calcd for $2C_9H_6N_2O_4 \cdot C_4H_8O_2$: C, 52.80; H, 4.02; N, 11.19. Found: C, 53.03; H, 4.13; N, 11.33.

2-Amino-5-carboxy-4-oxo-3,1,4-benzoxazine (13) was prepared in the same way as **11** and **12** and recrystallized from dioxane. On heating it decomposed in the range 205–210°.

Anal. Calcd for $C_9H_6N_2O_4$: C, 52.43; H, 2.93; N, 13.59. Found: C, 52.32; H, 2.88; N, 13.49.

The infrared spectra of **12** and **13** were similar to that of **9**, particularly with respect to the multiple absorption pattern in the 1600–1800-cm^{-1} region.

Products. The course of the reactions of **11**, **12**, and **13** studied kinetically were assumed to be the same as those of the parent compound **5**.[10,11] Their kinetic behavior supported this assumption as did the spectral characteristics of all intermediates and products. In particular, the addition of **11** to a methanolic solution of sodium methoxide followed by dilution with ether yielded a colorless salt whose infrared spectrum exhibited strong absorption at 2100 cm^{-1} (–C≡N or –N=C=N–, *i.e.*, **11e**).

Physical Methods. Kinetic and thermodynamic measurements were carried out exactly as recorded in a previous publication dealing with **5**.[10] Rate constants were obtained at constant pH but in the absence of added buffer by employing a spectrophotometric pH-Stat.[14]

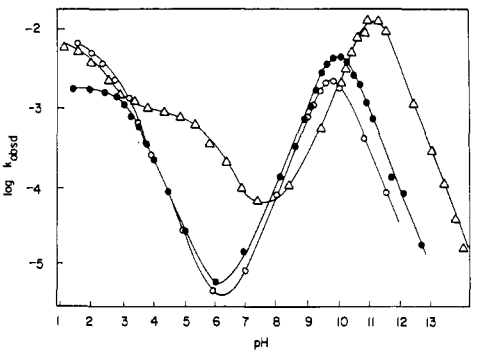


Figure 1. Plots of log k_{obsd} (k_{obsd} in sec^{-1}) *vs.* pH for the hydrolyses of compounds **5** (●), **11** (△), and **12** (○). Points are experimental and the curves generated from eq 1.

Results

Hydrolyses of **11** and **12** were followed spectrophotometrically (at both 270 nm and 360 nm) at constant pH in the absence of buffer species (30°, μ = 1.0 with KCl). The hydrolysis of **13** was carried out under the same conditions and followed at 265 nm. Plots of the log of the observed first-order rate constants (k_{obsd}) for **11** and **12** *vs.* pH are provided in Figure 1 along with the previously determined log k_{obsd}–pH

(7) F. Kurzen and K. Douraghi-Zadeh, *Chem. Rev.*, 67, 107 (1967).
(8) (a) M. Stiles and H. L. Finkbeiner, *J. Amer. Chem. Soc.*, 81, 505 (1959); (b) H. L. Finkbeiner and M. Stiles, *ibid.*, 85, 616 (1963).
(9) D. Y. Curtin and L. L. Miller, *Tetrahedron Lett.*, No. 23, 1869 (1965).
(10) Part I: A. F. Hegarty and T. C. Bruice, *J. Amer. Chem. Soc.*, 92, 6561 (1970).
(11) Part II: A. F. Hegarty and T. C. Bruice, *ibid.*, 92, 6568 (1970).
(12) C. W. James, J. Kenner, and W. V. Stubbings, *J. Chem. Soc.*, 117, 774 (1920).
(13) A. W. Hoffman, *Ber.*, 9, 1300 (1876).
(14) T. C. Bruice and J. R. Maley, *Anal. Biochem.*, 34, 275 (1970).

Table I. Empirical Parameters Describing the Rates of Hydrolysis of Compounds 5,[10] 11, 12, and 13

	5	11	12	13
k_1', sec^{-1}	1.70×10^{-3}	5.7×10^{-3}	6.4×10^{-3}	5.0×10^{-3}
k_2', sec^{-1}	3.10×10^{-2}	1.18×10^{-1}	1.35×10^{-2}	2.0×10^{-3}
k_3', sec^{-1}	2.40×10^{-6}	5.0×10^{-5}	2.0×10^{-6}	3.0×10^{-6}
k_4', sec^{-1}		8.4×10^{-4}	1.0×10^{-4}	4.0×10^{-5}
pK_{a_1}	3.10	1.65	2.44	2.28
pK_{a_2}	10.61	11.36	10.22	9.35
pK_{a_3}	9.21	10.00	9.30	8.20
pK_{a_4}		5.28	4.0	4.0

profile for 5.[10] The plot for 13 is not shown but is of the same form as the others.

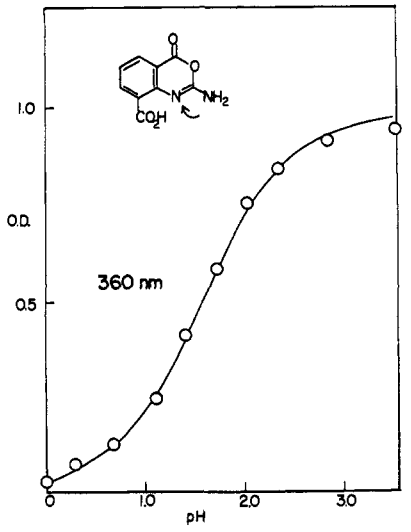


Figure 2. Spectrophotometric titration curve for pK_{a_1} of compound 11. The points are experimental and the curve theoretical.

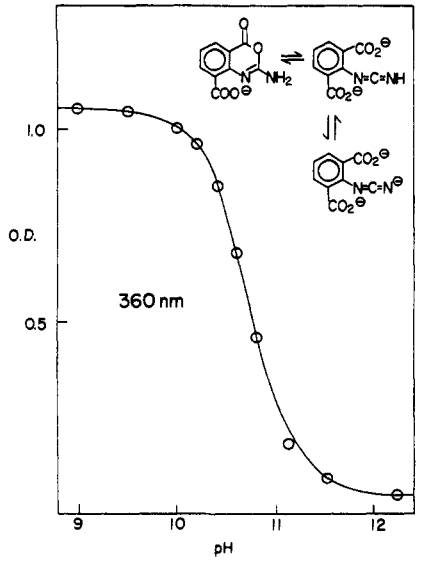


Figure 3. Spectrophotometric titration curve for pK_{a_2} and pK_{a_3} of compound 11. The points are experimental and the curve theoretical.

The curves drawn through the experimental points were generated from the empirical rate equation

$$k_{obsd} = \frac{k_1' a_H}{K_{a_1} + a_H} + \frac{k_4' a_H}{K_{a_4} + a_H} + k_3' + \frac{k_2' K_{a_2} a_H}{K_{a_2} K_{a_3} + K_{a_2} a_H + a_H^2} \quad (1)$$

The parameters yielding curves of best fit to the experimental points are reported in Table I.

The pK_a values employed to fit the log k_{obsd}–pH profile for 11, 12, and 13 to eq 1 were determined spectrophotometrically as previously described for 5.[10] In the case of pK_{a_4} of compound 11 a value of 5.28 provided a better fit of eq 1 than did the spectrophotometric value of 5.20. For pK_{a_1}, pK_{a_2}, and pK_{a_3} for 11 spectrophotometric titration was carried out at 360 nm while pK_{a_4} was determined at 298 nm. The spectrophotometric titration curves for 11 are representative and are provided in Figures 2, 3, and 4. The pK_{a_4} value for 13 is an apparent value from the kinetic data only since a suitable wavelength could not be found in this case for spectrophotometric titration.

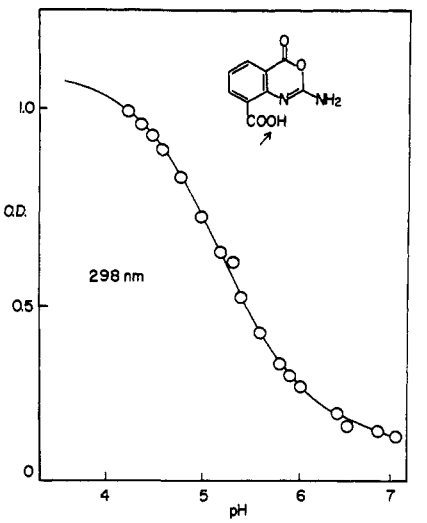


Figure 4. Spectrophotometric titration curve for pK_{a_4} of compound 11. The points are experimental and the curve theoretical.

The kinetic solvent deuterium isotope effect upon the hydrolysis of 11 at pD = pH = 4.0 was found to be k_{obsd}^H/k_{obsd}^D ($=8.32 \times 10^{-4}/4.25 \times 10^{-4}$) = 1.95, and at pD = pH = 4.38, k_{obsd}^H/k_{obsd}^D ($=7.60 \times 10^{-4}/4.03 \times 10^{-4}$) = 1.89.

The effects of metal ions on the hydrolysis rate of 11 were also briefly studied. Ratios of hydrolytic rate constants in the presence to those in the absence of added metal ions (0.1 *M*) were found to be 12 for Zn^{2+} at pH 6, 1 for Mg^{2+} at pH 6, 0.75 for Ba^{2+} at pH 6, 1.54 for Mn^{2+} at pH 6.1, 0.1 for Cu^{2+} at pH 4, 8 for Co^{2+} at pH 7, and 1 for Co^{2+} at pH 6.2.

Morpholinolysis of 11 was investigated over the pH range of 4.0–8.0 (30°, absence of added buffer, μ = 1.0 with KCl). Pseudo-first-order rate constants (k_{NT}), calculated on the basis of total morpholine concentra-

tion, for the disappearance of **11** in the presence of 1.0 *M* morpholine were found to be *ca.* 100-fold greater than in the presence of 0.01 *M* morpholine, as expected for a mechanism first order in free morpholine as found for the morpholinolysis of **5**.[11] The data obtained at 1.0 *M* total morpholine are presented in Table II along

Table II. Morpholinolysis of **11**[a]

pH	k_{NT}, sec^{-1}	k_{calcd}, sec^{-1}
4.0	0.120	0.117
5.0	0.750	0.760
6.0	1.65	1.70
7.0	2.00	1.90
8.0	1.65	1.60

[a] At 1.0 *M* total morpholine concentration hydrolysis does not contribute significantly to the disappearance of **11**.

with the calculated rate constants obtained employing eq 2. In eq 2 k_N is a second-order rate constant for

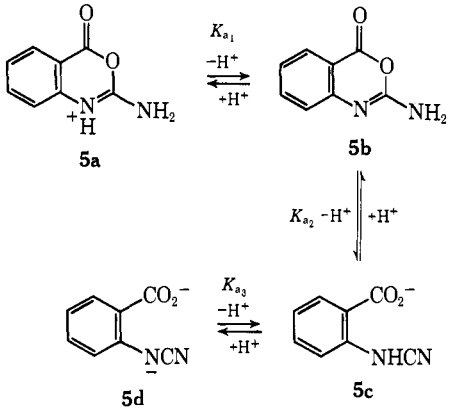
$$k_{NT} = \frac{k_N K_M a_H}{(K_{a_4} + a_H)(K_M + a_H)} \quad (2)$$

reaction of free morpholine with **11** (6.5×10^3 sec^{-1} M^{-1}), K_M the acid dissociation constant for protonated morpholine ($pK_M = 8.68$), and K_{a_4} that constant accompanying eq 1.

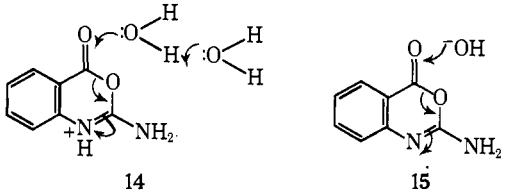
Discussion

The dissociation equilibria of **5** between pH 1 and 12 are provided in Scheme I.[10,11] Species **5d** and **5c** are

Scheme I

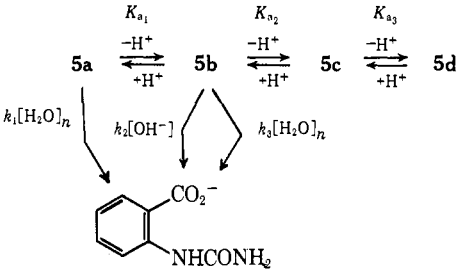


stable to nucleophilic attack by amine and lyate species. At low pH the sigmoid shape of the log k_{obsd}–pH profile is due to the spontaneous hydrolysis of **5a** (**14**).



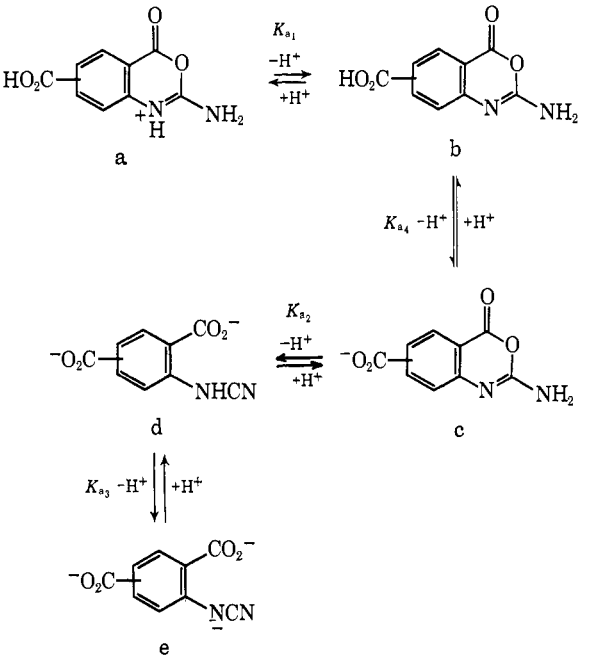
The bell-shaped portion of the profile is accounted for by hydroxide ion attack on **5b** (**15**) (the portion rising with pH) and conversion of **5b** to **5d** through **5c** (descending portion). Since $pK_{a_3} > pK_{a_2}$ conversion of **5b** to **5d** involves essentially the simultaneous loss of two protons, there being no more than 10% **5c** present at any pH. A small contribution of spontaneous hydrolysis of **5b** or the kinetically equivalent reaction of **5a** with hydroxide ion must be assumed (k_3') to provide a proper fit of the log k_{obsd}–pH profile. Hydrolysis of **5** is then accounted for by Scheme II. The reader is referred to ref 10 and 11 for confirmation of Schemes I and II.

Scheme II

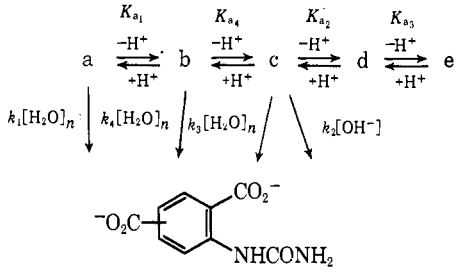


From the known hydrolytic scheme for **5**, those for **11**, **12**, and **13** are best portrayed as in Schemes III and IV.

Scheme III



Scheme IV



The rate constants for Scheme II have been derived from the empirical constants of Table I by the equations

$$k_1 = k_1'/[H_2O] \quad (3)$$

$$k_2 = k_2' K_{a_2}/K_w \quad (4)$$

$$k_3 = k_3'/[H_2O] \quad (5)$$

and for Scheme IV by eq 3, 4, 5, and 6. These derived constants are presented in Table III.

$$k_4 = k_4'/[H_2O] \quad (6)$$

Table III. Derived Rate Constants[a] for the Hydrolysis of Compounds **5**, **11**, **12**, and **13**

Compd no.	k_1	k_2	k_3	k_4
5	3.06×10^{-5}	51.5	4.3×10^{-8}	
11	1.04×10^{-4}	35	9.0×10^{-7}	1.51×10^{-5}
12	1.15×10^{-4}	55	3.6×10^{-8}	1.80×10^{-6}
13	9.0×10^{-5}	60.4	5.4×10^{-8}	7.17×10^{-7}

[a] Units are $sec^{-1}\ M^{-1}$.

In Schemes II and IV, k_1 has been interpreted as arising from attack of water on the ring nitrogen protonated species A. This is supported, in the case of **5**, by the kinetic solvent deuterium isotope effect of 2.0 for k_1.[10] Values of k_1 for **11**, **12**, and **13** are of similar magnitude and are all somewhat larger than that for **5**, as would be expected from the electronic effect of a protonated carboxyl group.[15]

For compound **5**, k_2 has been interpreted in terms of hydroxide ion attack on **5b**.[10] The k_2 values for **11**, **12**, and **13**, when considered in the same way, are very close in magnitude to the k_2 value for **5**. This presumably reflects both the small electronic effect of ionized carboxyl groups[15] and the absence of any significant electrostatic inhibition of the attack of the negatively charged hydroxide ion by the negatively charged carboxylate groups.

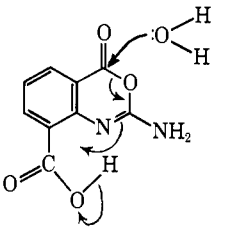
The constant k_4 for **11**, **12**, and **13** is given in Scheme IV as arising from attack of water on species b when both the carboxyl group and the ring nitrogen atom are uncharged. Second-order rate constants (k_4'') for the kinetically equivalent process, attack of hydroxide ion on the a species, are given by eq 7. Values of k_4'' are

$$k_4'' = k_4'K_{a_1}/K_w \text{ (or } k_4'' = k_3'K_{a_1}/K_w \text{ for } \mathbf{5}) \quad (7)$$

1.3×10^5, 1.27×10^9, 2.45×10^7, and 1.41×10^7 $sec^{-1}\ M^{-1}$ for **5**, **11**, **12**, and **13**, respectively. The magnitude and variation of these values make it seem unlikely that this interpretation of k_4' is correct, for **11** at least. The kinetic solvent deuterium isotope effect of *ca.* 1.9 for the hydrolysis of **11** in the pH range 4.0–4.4 is ambiguous and cannot be used to distinguish between the alternate mechanisms.

The value of k_4 for **11** is apparently some ten times greater than the corresponding values for **12** and **13**. It should be noted, however, that the values of k_4 for **12** and **13** can be accepted as upper limits only. The values of the rate and equilibrium constants for **12** and **13** lead to a situation (Figure 1) where the contribution from k_4 is almost overwhelmed by the k_1 contribution; consequently the k_4 values for these compounds must be regarded as rather uncertain. There is no problem in the case of **11** since both k_4 and pK_{a_4} are larger than for **12** or **13** and a clear plateau is observed.

It appears, however, that the presence of a neutral carboxyl group at the 8 position of the benzoxazine ring, *i.e.*, ortho to the ring nitrogen atom, promotes attack of water at the carbonyl group of **11b** when a comparison is made with the apparent rates of water attack on **12b** and **13b**. This is probably a result of intramolecular general acid catalysis (**16**). That the carboxyl group of **11b** is suitably placed for such catalysis is indicated by a comparison of the pK_{a_1} and pK_{a_4} values of **11**, **12**, and **13**. pK_{a_1} for **11** is some 0.7 unit below the pK_{a_1} values of **12** and **13**, and pK_{a_4} for **11** is 1.2 units above the corresponding values for **12** and **13**. This is strongly suggestive of hydrogen bonding between the un-ionized carboxyl group and the ring nitrogen atom of **11b**, even in aqueous solution. Benkovic and Dunikoski[16] have discussed the interdependence of intramolecular hydrogen bonding and intramolecular general catalysis.



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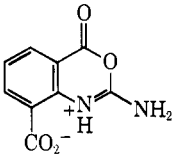
An alternative explanation for the apparent higher reactivity of **11b** is that it may arise simply as an electronic effect associated with a change in ρ or the susceptibility of the reaction to electronic substituent effects after a proton has been removed from the ring nitrogen atom. This, however, does not seem likely in view of the close similarity between the k_1 and k_2 values for **11**, **12**, and **13**. The effectiveness of the proposed catalysis of morpholinolysis, discussed below, also militates against an explanation in terms of a simple change in electronic substituent effects.

Another criticism which might be leveled at the conclusion of intramolecular catalysis, and one which deserves careful attention, is that the reactivity of **11b** may be associated with the zwitterionic species **11f**, which of course has the advantage, as does **11a**, of a fully protonated leaving group. Conclusive evidence that the predominant neutral form of **11** is not the zwitterion (*i.e.*, pK_{a_1} is as assigned in Scheme III) comes from the spectral data. The spectra of **5b**, **11b**, and **11c** are similar, in particular possessing a λ_{max} at 340 nm whereas **5a** exhibits only a trailing absorption at this wavelength. This absorption is employed in the spectrophotometric determination of pK_{a_1} at 360 nm (Figure 2). Extension of this titration to higher pH leads to no further significant increase in optical density. This shows that **11b** is essentially the only product of the first dissociation, because any extensive formation of the zwitterion **11f** would lead to an increase in absorption at 360 nm at higher pH when the protonated oxazine



11f

(15) J. E. Leffler and E. Grunwald, "Rates and Equilibria of Organic Reactions," Wiley, New York, N. Y., 1963, p 172.

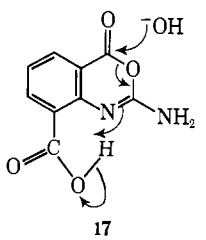
(16) S. J. Benkovic and L. K. Dunikoski, Jr., *Biochemistry*, 9, 1390 (1970).

ring of **11f** dissociated. The extinction coefficient of **11b** (and **11c**) at 360 nm was determined at pH 4–7 as 7400 (*cf.* that of **5b** of 4600). It can be estimated on the basis of the spectral data discussed above that no more than 5% of the zwitterion can arise from the first dissociation and this is an insufficient quantity to explain the observed reactivity unless **11f** were unusually reactive. Furthermore, the effects of a reactive zwitterion might be expected in the k_4 values of **12** and **13** to a similar extent as in **11**. This is apparently not so and hence the explanation of a reactive zwitterion for the enhanced reactivity of **11b** has been rejected. It is possible that the k_4 values of **12** and **13** could involve contributions from zwitterionic reactivity.

The effectiveness of the proposed intramolecular general acid catalysis of hydrolysis of **11b** can be gauged by comparison of k_4 for **11** with k_1 for **5**, **11**, **12**, and **13**. The reactivity of **11b** toward water is only a few times less than those of **5a**, **11a**, **12a**, and **13a**, where the leaving groups are fully protonated. This effectiveness is seen perhaps more dramatically in the results of the morpholinolysis of **11**.

Equation 2 shows that the reactive species of **11** with morpholine is **11b**. The second-order rate constant for reaction of this species with free morpholine is 6×10^3 sec^{-1} M^{-1}. In the case of **5**, free morpholine reacts with **5a** and **5b** with second-order rate constants of 1.6×10^4 and 6.3×10^{-2} sec^{-1} M^{-1}, respectively.[11] The reaction of morpholine with **11b** is thus approximately 10^5 times as rapid as with **5b**, and is almost as fast as the reaction with **5a** where the leaving group is protonated. The similarity of the rates of reaction of **5a**, **11a**, and **11b** with nucleophiles suggests that in the transition state pertaining to nucleophilic attack on the carbonyl group of **11b** the carboxyl proton is substantially transferred onto the ring nitrogen atom, whose basicity must increase considerably as the transition state is approached.[17]

Although k_3 values have been provided in Table III, their rationalization must be approached with some diffidence. The k_3' term of eq 1 whence they were derived strongly influences perhaps one point in the experimental log k_{obsd}–pH profile for each compound. Hence the pH dependence and in fact actual magnitude of this term are not reliably known. The figures as they stand suggest that the reactivity of water with **11c** is much greater than would be expected on the basis of comparison with that with **12c** and **13c**. There seems no obvious reason for this except, perhaps, in terms of the theory discussed above, and by invoking the kinetically equivalent mechanism, that the reaction of hydroxide ion with **11b** may be aided by intramolecular general acid catalysis as in **17**. The second-order rate



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constant for this reaction is given by eq 8 and has a

$$k_3'' = k_3' K_{a_4}/K_w \quad (8)$$

value of 1.8×10^4 sec^{-1} M^{-1}. Caution is needed with this interpretation, however, since the k_3'' values of **12** and **13**, also calculated from eq 8, are of comparable size, 1.35×10^4 and 2.0×10^4 sec^{-1} M^{-1}, respectively, and this without the advantage of the proposed intramolecular catalysis. It is not impossible, however, that the carboxyl group of **11** could catalyze the attack of water and morpholine but not that of hydroxide ion. In the transition state for hydroxide ion attack considerably less bond formation between nucleophile and substrate would be expected than for water or morpholine attack.[18] Consequently development of negative charge on the benzoxazine ring nitrogen atom would be much less and the stabilization of this charge by the adjacent carboxyl group much less important in the case of the transition state for hydroxide ion attack, leading to a reduction in or even to a disappearance of the intramolecular general acid catalysis observed with weaker nucleophiles. Holmquist and Bruice, for instance, have shown[19] that the presence or absence of stabilization or destabilization of transition states by electrostatic interactions between charged nucleophiles and charged esters depends on the strength of the nucleophile, *i.e.*, on the extent of bond formation in the transition state; the attack of weak oxygen anion nucleophiles on positively charged esters is enhanced over neutral esters but the attack of hydroxide ion is not.

There remains the further possibility that k_3 for **12** and **13** applies to water attack on **12c** and **13c**, respectively, and a term of similar size applies for **11c** but that the majority of the observed k_3 term for **11** is derived from k_3''. There is, in fact, some support for this idea in that k_3 for both **12** and **13** is very similar in magnitude to k_3 for **5**, just as the k_2 values are very close. A difference of nearly 300-fold between the rate of hydroxide attack on **12b** or **13b** (k_3'') and that on **12c** or **13c** (k_2) resulting only from ionization of the carboxyl group seems unreasonably large. This also suggests that the interpretation of k_3 for **12** and **13** (and **5**) as water attack on the c (or b for **5**) species is correct. All of this, however, is speculation, and in the absence of further evidence, probably unprofitable speculation.

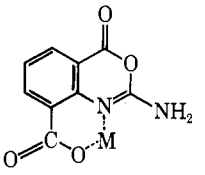
Another feature of the experimental results not easily explicable with the data on hand is the variation of pK_{a_2} and pK_{a_3} among the compounds studied. The values for **12** are similar to those for **5** as would be expected since the only difference between the species involved is the carboxylate group on the far side of the benzoxazine molecule from the ionization site. The changes seen with **11** and **13** are not easily rationalized. The increase in pK_{a_2} and pK_{a_3} on progression from **5** to **11** could possibly be explained on electrostatic grounds, but such an explanation should also apply to **13** where in fact the pK_a shift is in the opposite direction. The situation is complicated by the oxazine ring opening which occurs during these ionizations.[10] A lack of knowledge of the synchronization and steric and electronic effects of substituents on this sequence of reactions probably precludes worthwhile speculation here.

(17) W. P. Jencks, "Catalysis in Chemistry and Enzymology," McGraw-Hill, New York, N. Y., 1969, pp 239–242.

(18) T. C. Bruice, A. Donzel, R. W. Huffman, and A. R. Butler, *J. Amer. Chem. Soc.*, **89**, 2106 (1967); W. P. Jencks and M. Gilchrist, *ibid.*, **90**, 2622 (1968); T. C. Bruice, A. F. Hegarty, S. M. Felton, A. Donzel, and N. G. Kundu, *ibid.*, **92**, 1370 (1970).

(19) B. Holmquist and T. C. Bruice, *J. Amer. Chem. Soc.*, **91**, 2985 (1969).

Given the intramolecular general acid catalysis mechanism for the hydrolysis of **11b** proposed above (**16**), it is perhaps surprising that metal ions do not have a more pronounced effect on the hydrolysis of **11**. The formation of a chelate complex such as **18** could be imagined, from which the reactivity of the a species associated with nitrogen protonation might be expected. The strangely varied results from an admittedly cursory examination of the hydrolysis of **11** in the presence of several metal ions, along with changes in the substrate spectrum in the presence of some of the metals (*e.g.*, Cu^{2+}), certainly point toward complex formation but these complexes clearly have no exceptional hydrolytic reactivity.



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Despite the uncertainties discussed in the preceding four paragraphs, the main point of this paper—that the reaction of nucleophiles with the carbonyl group of 2-amino-4-oxo-3,1,4-benzoxazine is strongly enhanced by addition of a neutral carboxyl group to the 8 position—seems indisputable. This enhancement is most likely an example of intramolecular general acid catalysis (**16**) involving proton donation by the carboxyl group to the ring nitrogen atom simultaneously with nucleophilic attack at the carbonyl group. The catalysis observed seems particularly efficient, the reactive species **11b** being only a few times less susceptible to nucleophilic attack than the species where the ring nitrogen atom is fully protonated. Of more importance perhaps is the fact that this catalysis ensures the reactivity of the benzoxazine system with nucleophiles at pH's just below neutrality where the basic system is inherently inert. Incorporation of a carboxyl group at the 5 position of the benzoxazine, ortho to the reactive carbonyl group (**13**), yields hydrolysis rates practically indistinguishable from those of a 6-carboxyl isomer (**12**) where participation is impossible. Thus the carboxyl at the 5 position apparently does not participate as a general acid, general base, or nucleophile to assist intermolecular nucleophilic attack.

The considerable advantage of a carboxyl group adjacent to an imidazoline nitrogen atom in biotin in terms of the proposed biotin mechanism (I) has thus been demonstrated.

Acknowledgment. This work was supported by a grant from the National Science Foundation.

The Hydrolysis of *N*-Aryl Carbamyl Phosphate Mono- and Dianions[1]

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Contribution from the Department of Biochemistry, College of Medicine, University of Florida, Gainesville, Florida 32601. *Received June 18, 1970*

Abstract: Pseudo-first-order rate constants for the hydrolysis of *N*-phenyl, *N*-*p*-ethoxyphenyl, *N*-*p*-nitrophenyl, and *N*-*m*-chlorophenyl carbamyl phosphate have been obtained at 37° and ionic strength 0.60 *M* over a pH range in which mono- and dianionic species predominate. The rates of hydrolysis of the monoanionic species are markedly less sensitive to the electron-delocalizing ability of para substituents than those found for the dianionic species. The effects of varying temperature, ionic strength, organic solvent, and buffer concentrations on the k_{obsd} values for both mono- and dianions are consistent with unimolecular reaction mechanisms. Methanolysis studies indicate that the monoanionic and dianionic species of the substituted carbamyl phosphates undergo primarily P–O bond fission. However, at least in the case of *p*-nitrophenyl carbamyl phosphate monoanion, some C–O bond fission also occurs. Azide trapping studies support this conclusion since it can be shown that the *p*-nitrophenyl carbamyl group cannot be trapped during *p*-nitrophenyl carbamyl phosphate dianion hydrolysis in 1 *M* azide, whereas some carbamyl trapping is observed during hydrolysis of the monoanion. The data presented here indicate that mono-substituted carbamyl phosphate monoanions may hydrolyze *via* more than one mechanism, one of which is similar to that for the monoanions of carbamyl phosphate and other acyl phosphates whereas the mono-substituted carbamyl phosphate dianions hydrolyze *via* a mechanism similar to that for other acyl phosphates but different from the unsubstituted carbamyl phosphate dianion.

Acyl phosphates have been studied with regard to the rates and mechanism of hydrolysis with a variety of results. Studies of the uncatalyzed hydrolysis of the aliphatic acyl[2,3] and benzoyl phosphates[2] have led to the general conclusion that the mechanisms for the mono- and dianionic species involve unimolecular elimination reactions, with P–O bond fission, resulting in the release of the free carboxylic acid or carboxylate anion with the formation of the postulated monomeric metaphosphate ion as an intermediate to inorganic phosphate formation.

The mechanism of hydrolysis of carbamyl phosphate and its substituted derivatives appears, however, to be

(1) We are grateful to the National Institute of Arthritis and Metabolic Disease (Grant No. AM 12193) and to the College of Medicine, University of Florida, for financial support.

(2) G. Di Sabato and W. P. Jencks, *J. Amer. Chem. Soc.*, **83**, 4400 (1961).

(3) D. R. Phillips and T. H. Fife, *J. Org. Chem.*, 34, 2710 (1969).