Hydrolysis of Imidate Esters Derived from Weakly Basic Amines. II. The Influence of General Acid–Base Catalysis on the Partitioning of Tetrahedral Intermediates[1a-c]

Tadashi Okuyama,[1d] David J. Sahn, and Gaston L. Schmir*

Contribution from the Department of Molecular Biophysics and Biochemistry, Yale University School of Medicine, New Haven, Connecticut 06510.
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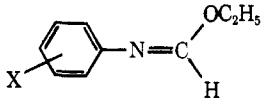
Abstract: The influence of general acid-base catalysts on the nature of the products of hydrolysis of imidate esters has been studied with five imidate esters (I–V) derived from aniline and nitro-substituted anilines. Depending upon the structure of the imidate esters, catalysts either increase the amine yield at constant pH (with I and II) or decrease the amine yield (with IV). With derivatives of *p*-nitroaniline (III and V), some catalysts (phosphate and acetate) increase the yield of amine while others (imidazole and pyridine) decrease the amine yield. These observations have been interpreted in terms of mechanisms involving interactions between the general acid-base catalysts and the tetrahedral addition intermediates formed by hydration of the imidate esters. In particular, it is suggested that the different modes of breakdown of the intermediates derived from the *p*-nitrophenyl imidates III and V reflect the different mechanisms of action of bifunctional and monofunctional catalysts.

The products of the hydrolysis of imidate esters vary with pH. In general, imidates are converted to amines and esters in acidic solution, and to amides and alcohols at alkaline pH,[1a,2-7] with the exception of imidates derived from very weakly basic amines ($pK_a < 0$), where the reverse is true.[1a] At constant pH, amine buffers cause modest increases in the yield of amine (and ester) formed on hydrolysis of imidate esters,[2,4] while potential bifunctional buffers (phosphate, and bicarbonate) at very low concentrations markedly increase the yield of amine.[3,4,7,8] With ethyl thioacetimidate[9] and *p*-tolyl *N*,*N*-dimethylacetimidate,[10] the increase in amine yield produced by carboxylate buffers has been interpreted in terms of a mechanism involving rate-determining proton transport processes.

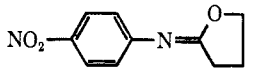
Our continuing interest in the properties of the tetrahedral addition intermediates involved in the hydrolysis of imidate esters and in many acyl transfer reactions[11] led us to investigate the effects of general acid–base catalysts on the hydrolysis of imidate esters wherein the basicity of the amine component was systematically decreased. We hoped thereby to obtain additional information on the interaction of tetrahedral intermediates with mono- and bifunctional general acid–base catalysts.

Results

The influence of buffers on the hydrolysis of four *N*-arylformimidates (I–IV) and an imino lactone (V)



I, H
II, *m*-NO_2
III, *p*-NO_2
IV, 2,4-dinitro



V

was investigated. Extensive kinetic data have previously been reported for the hydrolysis of these compounds,[1a] and the present study concerns itself principally with the relationship of the type and concentration of buffer species to the nature of the products of hydrolysis.

The remarkable diversity of the buffer effects encountered with these substrates is illustrated in Figures 1–3. With the *m*-nitro imidate II, buffers generally increase the yield of *m*-nitroaniline, although, not surprisingly, major differences exist between various buffers in their ability to promote the formation of amine (Figure 1). In the case of the *p*-nitrophenyl derivative III, the yield of amine is efficiently increased by phosphate buffer, and less dramatically by acetate buffers. On the other hand, pyridine and imidazole buffers *decrease* the yields of *p*-nitroaniline (Figure 2). The response of the *p*-nitrophenyl imino lactone V is qualitatively the same as that of III. Finally, *all buffers examined* (including phosphate and acetate) *lead to a decrease in the formation of 2,4-dinitroaniline from IV* (Figure 3).

The increase or decrease in amine yield formed in imidate hydrolysis in the presence of a catalytic buffer is described by a rectangular hyperbola, according to eq 1

$$\Delta A/\Delta A_{max} = [\text{buffer}]/([\text{buffer}] + K_{app}) \qquad (1)$$

(ΔA = increase or decrease in amine yield as compared with yield at zero buffer concentration; ΔA_{max} = maximum increase or decrease possible; K_{app} = concentration of buffer required to produce half the maximum possible increase or decrease in yield).[4,8,9] For experiments with very effective catalysts (*e.g.*, phosphate), low concentrations of relatively unreactive

(1) (a) Part I: T. Okuyama, T. C. Pletcher, D. J. Sahn, and G. L. Schmir, *J. Amer. Chem. Soc.*, **95**, 1253 (1973). (b) This work is taken in part from a dissertation presented by D. J. S. in partial fulfillment of the requirements for the M. D. degree, Yale University, 1969. (c) Financial support by the National Institutes of Health and the National Science Foundation is gratefully acknowledged. (d) On leave from the faculty of Engineering Science, Osaka University.

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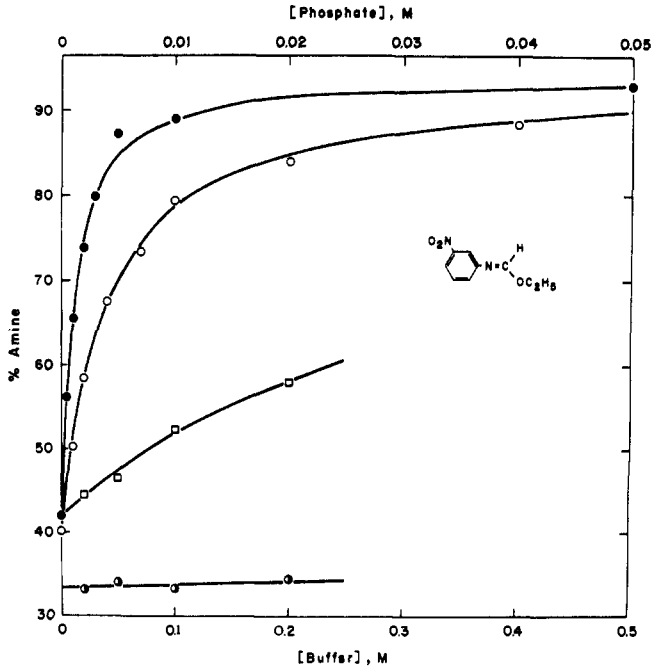


Figure 1. Effect of buffers on yield of amine obtained from hydrolysis of II: ●, phosphate, pH 6.11 (abscissa, upper scale); ○, acetate, pH 6.13; □, pyridine, pH 6.18; half-shaded circle, imidazole, pH 6.20. All the phosphate and acetate reaction mixtures contain 0.01 *M* MES buffer. The curves for phosphate and acetate are calculated from eq 1, using the values of K_{app} given in Table III.

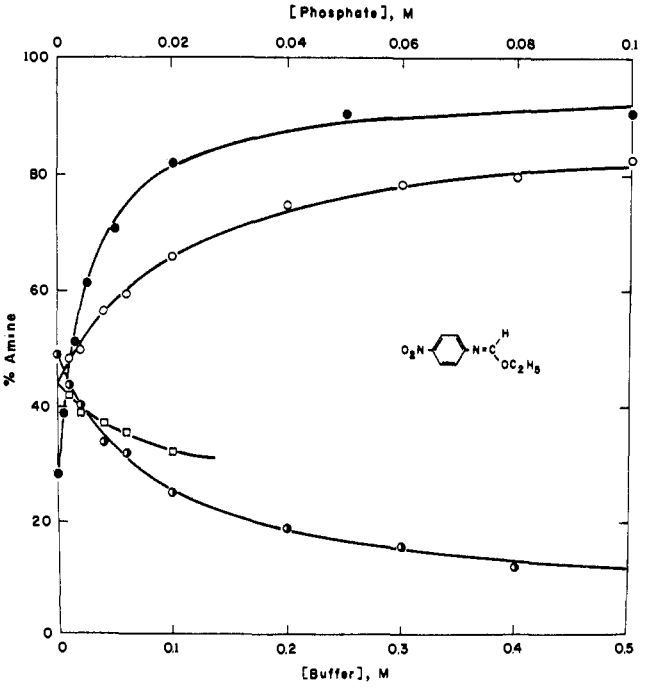


Figure 2. Effect of buffers on yield of amine obtained from hydrolysis of III: ●, phosphate, pH 5.91 (abscissa, upper scale); ○, acetate, pH 5.75; □, pyridine, pH 5.75; half-shaded circle, imidazole, pH 5.75. All the phosphate and imidazole reaction mixtures contain 0.01 *M* MES buffer. Curves are calculated from eq 1, using the values of K_{app} given in Table III.

buffers were used to maintain constant pH. Relative abilities of various buffers to alter the yield of amine formed in these reactions are expressed by the values of K_{app}, which were calculated by computer fitting of the data to the equation for the two-parameter rectangular

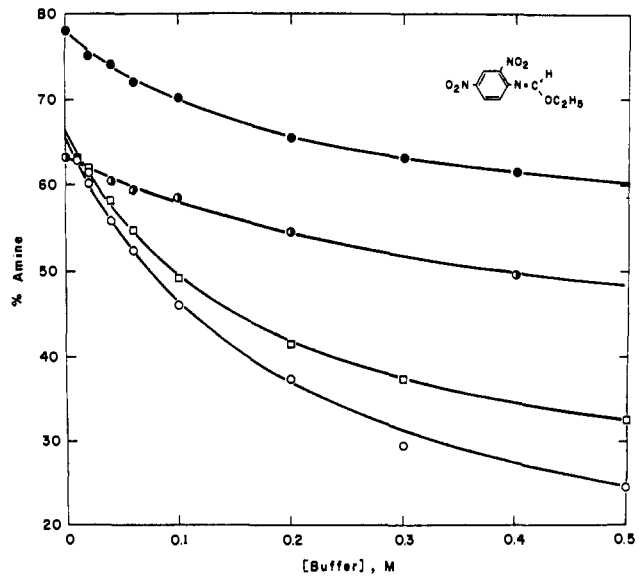


Figure 3. Effect of buffers on the yield of amine obtained from hydrolysis of IV: ●, phosphate, pH 5.38; half-shaded circle, imidazole, pH 4.95; □, pyridine, pH 4.96; ○, acetate, pH 4.98. All the phosphate and imidazole reaction mixtures contain 0.01 *M* pyridine buffer. Curves are calculated from eq 1 using the values of K_{app} given in Table III.

hyperbola[12] (when a measurement at zero buffer concentration could be made, *i.e.*, in the presence of an unreactive buffer), or to the three-parameter hyperbola,[13] which also yielded an estimate of the yield at zero buffer concentration.

In earlier work,[4,8] striking differences had been noted in the abilities of phosphate and imidazole buffers to increase the yield of amine in the hydrolysis of imidate (but not thioimidate)[14] esters. These differences also are seen with formimidates I–III, and with V (Table I). With the unsubstituted imidate I, phosphate is about 100 times more effective than imidazole in catalyzing the formation of aniline. As electron withdrawal in the aniline moiety increases, phosphate becomes somewhat less effective, but imidazole either has essentially no influence on amine yield (with the *m*-nitro imidate II) or decreases the yield of *p*-nitroaniline (with III and V). In the case of the least basic substrate IV, both phosphate and imidazole buffer decrease amine yield, with about the same effectiveness (compare K_{app} values), but not to the same limiting value.

Amine buffers differ in the extent to which they produce an increase in the conversion of I to aniline. The effects of some tertiary amines on the hydrolysis of I at pH about 7 are compared in Table II. Owing to the weak catalysis by these buffers, amine yield increases approximately linearly with buffer concentration; K_{app} values were not calculated.[15]

(12) K. R. Hanson, R. Ling, and E. Havir, *Biochem. Biophys. Res. Commun.*, **29**, 194 (1967).
(13) W. W. Cleland, *Nature (London)*, **198**, 463 (1963).
(14) R. K. Chaturvedi, A. E. MacMahon, and G. L. Schmir, *J. Amer. Chem. Soc.*, **89**, 6984 (1967).
(15) (a) We have previously suggested[15b] that values of K_{app} could be obtained directly from the initial slopes of plots of amine yield *vs.* buffer concentration. This approximation is true only if, in eq 1, ΔA_{max} is essentially equal to unity, *i.e.*, amine yield at zero buffer concentration is nearly zero and amine yield at high buffer concentration is about 100%. In the general situation where this may not be true, the initial slope = $\Delta A_{max}/K_{app}$, and not $1/K_{app}$, as previously stated. (b) G. L. Schmir, *J. Amer. Chem. Soc.*, **90**, 3478 (1968).

Table I. Effect of Phosphate and Imidazole Buffers on the Yield of Amine Obtained from Hydrolysis of Formimidates I–IV[a]

Expt	pH	Buffer	Concn, M	Amine yield, %	Intercept,[b] %	Maximum or minimum yield,[c] %	10^3K_{app}, M[d]
			Ethyl *N*-Phenylformimidate (I)				
1[e]	6.57	Phosphate	0.0	46.5	46.5	89.9 ± 0.5	0.4 ± 0.02
	6.53		0.0001	55.1			
	6.56		0.0005	69.9			
	6.56		0.001	77.7			
	6.53		0.01	88.9			
2	6.48	Imidazole	0.01	56.3	49.4 ± 0.7	86.4	42 ± 3
	6.48		0.04	66.9			
	6.48		0.10	75.4			
	6.45		0.40	82.8			
			Ethyl *N*-*m*-Nitrophenylformimidate (II)				
3[e]	6.14	Phosphate	0.0	42.0	42.0	94.4 ± 0.8	1.2 ± 0.1
	6.13		0.0004	56.1			
	6.12		0.002	73.8			
	6.12		0.005	87.3			
	6.08		0.05	93.1			
4	6.21	Imidazole	0.02	33.1	33.1		
	6.20		0.05	34.0			
	6.20		0.10	33.3			
	6.19		0.20	34.6			
			Ethyl *N*-*p*-Nitrophenylformimidate (III)				
5[e]	5.68	Phosphate	0.0	49.0	49.0	95.4 ± 0.4	5.8 ± 3
	5.69		0.003	63.9			
	5.71		0.01	77.7			
	5.75		0.05	90.0			
6[e]	5.68	Imidazole	0.0	49.0	49.0	5.9 ± 1.2	82 ± 7
	5.74		0.02	40.4			
	5.76		0.06	32.0			
	5.79		0.20	19.0			
	5.76		0.40	12.3			
			Ethyl *N*-2,4-Dinitrophenylformimidate (IV)				
7[f]	4.97	Phosphate	0.0	63.2	63.2	15.9 ± 1.8	260 ± 20
	5.06		0.04	57.2			
	5.10		0.10	49.9			
	5.14		0.30	37.7			
8[f]	4.97	Imidazole	0.0	63.2	63.2	34.9 ± 4.1	440 ± 110
	4.96		0.04	60.4			
	4.95		0.10	58.5			
	4.91		0.40	49.6			
			2-(*N*-*p*-Nitrophenyl)iminotetrahydrofuran (V)				
9[g]	6.10	Phosphate	0.0	39.8	39.8	84.8 ± 0.7	13 ± 0.7
	6.16		0.005	52.4			
	6.16		0.02	67.2			
	6.16		0.12	80.2			
10	6.08	Imidazole	0.02	58.0	61.0		
	6.06		0.06	53.4			
	6.05		0.10	50.3			

[a] At 30°, in 2% CH_3CN–H_2O (I–IV), or 10% CH_3CN–H_2O (V); μ = 0.5. [b] Yield at zero buffer concentration, measured directly in the presence of an unreactive buffer, or extrapolated. [c] Extrapolated value at infinite buffer concentration, with standard deviation. [d] Standard deviation is indicated. [e] All solutions contain 0.01 M 2-*N*-morpholinoethanesulfonate buffer. [f] All solutions contain 0.01 M pyridine buffer. [g] All solutions contain 0.03 M imidazole buffer.

With each of the formimidates I–IV, the effect of several monofunctional and (potential) bifunctional buffers on product nature and yield was investigated, in some cases at a number of different pH values. The results of these experiments are summarized in Table III. Some salient aspects of these data are as follows. Phosphate buffers effectively increase amine yield (with I–III), but the maximum yields of amine reached asymptotically at high buffer concentration decrease with increasing pH (experiments 21–27 and Figure 4). With the *p*-nitro imidate III, the variation of these limiting values with pH is reasonably well correlated with the simple sigmoid curve characteristic of the ionization of a monovalent acid (Figure 5), of apparent pK = 7.16 and with asymptotes at 99% (low pH) and 27% (high pH). Although this tendency to lower maximum yields at high pH is best seen with III, it is also indicated by the data for the *m*-nitro imidate (experiments 14–18) and the iminolactone (experiments 43–46).

The catalytic effectiveness of phosphate buffers *decreases* rapidly with increasing pH when this buffer increases amine yield (*i.e.*, with I–III and V). The response of imidazole buffers to increasing pH is not consistent. With the unsubstituted imidate I, there is a suggestion that imidazole is maximally effective at about pH 6.7, while with the *p*-nitro imidate III, catalysis by imidazole *increases* with increasing pH. Insufficient data are available with other buffers to allow firm conclusions concerning the effects of pH on their reactivity, although it is clear that acetate buffers become less

Table II. Effect of Amine Buffers on the Yield of Aniline Obtained from Hydrolysis of Ethyl *N*-Phenylformimidate (I)[a]

Expt	pH	Buffer (pK_a)[b]	Concn, *M*	Aniline yield, %	Slope[c] (mole fraction M^{-1})	Slope/f[d]
1	6.88	MES[e]	0.005	18.3	2.0	12.8
	6.89	(6.16)	0.02	24.9		
	6.90		0.06	31.2		
	6.90		0.10	38.0		
2	7.04	Imidazole	0.01	24.4[f]	8.1	16.4
	7.03	(7.02)	0.02	32.9		
	7.03		0.06	48.3		
	7.02		0.10	55.1		
3	6.98	β-Dimethylaminopropionitrile	0.005	15.6	2.55	4.1
	7.01	(7.22)	0.01	16.5		
	7.01		0.02	19.8		
	7.02		0.04	25.3		
	7.02		0.06	28.7		
4	6.88	*N*-Methylmorpholine	0.01	21.9	1.30	1.5
	6.89	(7.59)	0.02	23.1		
	6.90		0.06	27.9		
	6.90		0.10	33.8		
5	7.27	*N*-Ethylmorpholine	0.01	8.0	0.55	0.69
	7.28	(7.88)	0.04	9.5		
	7.28		0.10	13.7		
	7.27		0.20	17.7		

[a] At 30°, in 2% CH_3CH–H_2O, μ = 0.5. [b] Approximate pK_a of amine buffer under conditions of experiment. [c] Initial slope of plots of mole fraction amine *vs.* concentration of buffer. [d] $f = [H^+]/([H^+] + K_a)$. [e] 2-*N*-Morpholinoethanesulfonic acid. [f] Extrapolated yield at zero buffer concentration is 16%.

effective as pH increases, both with II and IV. Finally, we note that when buffers decrease the yield of amine from III and IV, minimum yields are very variable, depending both on the nature of the buffer and, for a given buffer, on pH.

Ultraviolet spectra (see Experimental Section) of reaction mixtures were taken after complete hydrolysis of the 2,4-dinitrophenyl imidate IV in phosphate (pH 6) and acetate (pH 5) buffers, and could be accounted for quantitatively in terms of mixtures of 2,4-dinitroaniline and 2,4-dinitroformanilide. The ultraviolet spectra of 2,4-dinitroaniline and 2,4-dinitrophenolate ion (but not 2,4-dinitrophenol) are rather similar; on acidification of the above reaction mixtures to pH 1, no change in spectrum occurred. These observations suggest that aromatic nucleophilic substitution does not take place during the hydrolysis of IV.

The rate of hydrolysis (5.6×10^{-3} sec^{-1}) of the *m*-nitro imidate II (0.01 *M* MES buffer, pH 6.5) is unchanged on addition of 0.03 *M* phosphate buffer. Under these conditions, the yield of *m*-nitroaniline increases from 10 to 84%. With the dinitroimidate IV, increasing the concentration of pyridine buffer from 0.02 to 0.5 *M* at pH 5 does not alter the rate (<2% change), while the yield of amine falls from 62 to 33%. The same increase in acetate buffer concentration increases the rate of hydrolysis by 25%, and the yield of dinitroaniline falls from 60 to 25%. To account for the changes in product distribution in terms of effects on the rates of parallel, competing reactions of IV, rate increases of 190 and 245% would have been required for the pyridine and acetate experiments, respectively. As with other imidate esters derived from more basic amines,[4,8] the effects of buffers on the products of hydrolysis of II and IV (and probably of the other imidates in this study) are largely independent of the buffer effects on the reaction rates, suggesting, once again, that the *rate-determining* step of the reaction precedes the *product-determining* step(s), and that the change in products is the result of interaction between buffer and one or more species of an intermediate.

Discussion

Strong evidence for the proposal that the hydrolysis of imidate esters I–V involves the participation of transient intermediates has been provided through the independent effects of pH on the rates and products of hydrolysis.[1a] Additional support for this conclusion is found in the buffer experiments cited above, in accord with general experience with imidate[4,7,8] and thioimidate[9,14] esters. Detailed studies of the influence of buffers on the yield of amine produced in the hydrolysis of an iminolactone[8] and acyclic imidate esters[4] led to the following conclusions. Phosphate mono- and dianion, bicarbonate ion, and probably also acetic acid interact with a neutral tetrahedral intermediate to promote the expulsion of amine. Tertiary amines also increase amine yield,[4] possibly by interaction of the conjugate acid of the amine with an anionic tetrahedral intermediate, although insufficient data were available to conclusively prove this point. It was suggested that the unusual efficiency of catalysts such as $H_2PO_4^-$, HPO_4^{2-}, HCO_3^-, and arsenate resided in their ability to effect a more or less concerted proton shift in the neutral intermediate; monofunctional catalysts such as imidazolium ion could not use this mechanism and were thus much less effective than bifunctional acids of the same p*K* (*e.g.*, phosphate).

The possible interactions between the tetrahedral intermediate and general acid–base catalysts appear to be more numerous and more complex than had been previously envisaged. Presumably, the ability of certain buffers to decrease amine yield results from an enhanced rate of expulsion of alcohol from the tetrahedral intermediate; similarly, the observation that, even though amine yield is increased, the maximum yield of amine is less than 100% and is pH dependent again suggests that, in certain cases, buffers are able to ac-

Table III. Effects of Buffers on the Yield of Amine Obtained from Hydrolysis of Imidate Esters I–V[a]

Expt	pH[b]	Buffer	Concn range, M	No. of points	Intercept,[c] %	Maximum or minimum yield, %[d]	K_{app}, M
			Ethyl *N*-Phenylformimidate (I)				
1[e]	6.55	Phosphate	0.0001–0.02	10	46.5	89.9 ± 0.5	$(4.1 \pm 0.2) \times 10^{-4}$
2[f]	6.84		0.0001–0.025	7	30.1	90.7 ± 0.7	$(7.1 \pm 0.4) \times 10^{-4}$
3[g]	7.37		0.0005–0.07	11	7.5	83.1 ± 1.2	$(3 \pm 0.2) \times 10^{-3}$
4[g]	8.00		0.002–0.15	9	3.5	80.3 ± 1.1	0.018 ± 0.001
5[h]	8.36		0.005–0.15	9	6.5	79.1 ± 1.8	0.049 ± 0.003
6	6.47	Imidazole	0.01–0.40	7	49.4 ± 0.7	86.4	0.042 ± 0.003
7	6.68		0.005–0.20	9	37.0 ± 1.0	80.3	0.032 ± 0.004
8	6.76		0.005–0.50	12	26.6 ± 1.5	82.5	0.032 ± 0.004
9	7.02		0.01–0.20	10	16.2 ± 2.3	83.3	0.064 ± 0.012
10	7.40		0.005–0.50	12	7.3 ± 0.9	75.5	0.091 ± 0.002
11	6.16	MES[i]	0.005–0.10	7	72.6 ± 1.1	85.6	0.042 ± 0.002
12	6.38		0.005–0.50	11	60.0 ± 1.3	92.6	0.084 ± 0.018
13	6.59		0.01–0.10	9	38.6 ± 1.4	84.2	0.099 ± 0.038
			Ethyl N-*m*-Nitrophenylformimidate (II)				
14[e]	6.11	Phosphate	0.0004–0.20	10	42.0	94.4 ± 0.8	$(1.2 \pm 0.1) \times 10^{-3}$
15[e]	6.52		0.0005–0.03	11	10.2	94.8 ± 1.3	$(3 \pm 0.2) \times 10^{-3}$
16[e]	7.12		0.002–0.10	11	0.4	86.9 ± 1.6	0.012 ± 0.001
17[e]	7.47		0.005–0.15	9	2.0	79.5 ± 1.1	0.040 ± 0.002
18[g]	7.88		0.005–0.15	9	2.2	66.9 ± 2.0	0.091 ± 0.006
19[e]	6.13	Acetate	0.01–0.40	8	40.2	93.5 ± 1.6	0.039 ± 0.004
20[e]	6.52		0.02–0.40	9	11.6	95.2 ± 2.2	0.18 ± 0.01
			Ethyl *N*-*p*-Nitrophenylformimidate (III)				
21[e]	5.72	Phosphate	0.001–0.20	9	49.0	94.5 ± 0.4	$(5.8 \pm 0.3) \times 10^{-3}$
22[e]	5.91		0.001–0.10	8	28.4	95.3 ± 1.1	$(5.3 \pm 0.4) \times 10^{-3}$
23[e]	6.19		0.002–0.20	8	12.3	91.9 ± 1.2	$(8.5 \pm 0.5) \times 10^{-3}$
24[e]	6.53		0.005–0.20	7	4.2	88.2 ± 1.8	0.019 ± 0.002
25[e]	7.09		0.01–0.20	7	4.4	66.5 ± 1.6	0.067 ± 0.005
26[g]	7.42		0.01–0.30[j]	10	2.0	47.8 ± 2.3	0.18 ± 0.02
27[g]	7.75		0.02–0.40[j]	8	1.6	44.5 ± 3.8	0.41 ± 0.06
28[e]	5.43	Imidazole	0.02–0.50	9	69.1	25.8 ± 2.2	0.19 ± 0.03
29[e]	5.75		0.01–0.40	9	49.0	5.9 ± 1.2	0.082 ± 0.007
30[e]	5.99		0.01–0.40	10	22.9	10.6 ± 0.6	0.037 ± 0.006
31	5.71	MES[i]	0.01–0.40	8	49.5 ± 0.3	43.5	0.12 ± 0.03
32	5.75	Pyridine	0.01–0.10	5	43.8 ± 1.2	22.8	0.085 ± 0.054
33	5.75	Acetate	0.01–0.50	9	43.9 ± 1.1	90.8	0.114 ± 0.015
			Ethyl *N*-2,4-Dinitrophenylformimidate (IV)				
34[k]	5.07	Phosphate	0.01–0.40	9	63.2	15.9 ± 1.8	0.26 ± 0.02
35[k]	5.38		0.02–0.40	8	78.1	52.8 ± 1.3	0.21 ± 0.03
36[e]	5.66		0.02–0.40	9	79.6	67.7 ± 0.8	0.17 ± 0.03
37[k]	4.95	Imidazole	0.02–0.40	7	63.2	34.9 ± 4.1	0.44 ± 0.11
38	4.96	Pyridine	0.01–0.50	8	66.3 ± 0.5	20.7	0.18 ± 0.02
39	5.35		0.01–0.50	9	78.9 ± 0.6	39.7	0.22 ± 0.03
40	4.98	Acetate	0.01–0.50	8	65.3 ± 0.2	8.1	0.20 ± 0.01
41	5.54		0.02–0.50	8	78.5 ± 0.2	−1.8	0.56 ± 0.03
			2-(*N*-*p*-Nitrophenyl)iminotetrahydrofuran (V)				
43[l]	6.15	Phosphate	0.001–0.25	9	39.8	84.8 ± 0.7	0.013 ± 0.0007
44[l]	6.31		0.001–0.25	9	26.1	77.1 ± 1.8	$(8.3 \pm 1) \times 10^{-3}$
45[l]	6.75		0.002–0.23	8	6.3	78.8 ± 2.1	0.026 ± 0.002
46[l]	7.54		0.002–0.50[m]	10	4.9	46.3 ± 0.8	0.120 ± 0.006

[a] At 30°, in 2% CH_3CN–H_2O, μ = 0.5, except when noted. [b] Average pH for set; average deviation is ≤0.03, except in 3 cases. [c] Yield at zero buffer concentration, either measured directly in the presence of an unreactive buffer, or extrapolated; when extrapolated, standard deviation is given. [d] Extrapolated value at infinite buffer concentration, with standard deviation. [e] In presence of 0.01 M MES buffer. [f] In presence of 0.02 M β-dimethylaminopropionitrile buffer. [g] In presence of 0.01 M β-dimethylaminopropionitrile buffer. [h] In presence of 0.01 M *N*-ethylmorpholine buffer. [i] 2-*N*-Morpholinoethanesulfonate. [j] For points at phosphate ≥0.2 M, μ exceeds 0.5 M. [k] In presence of 0.01 M pyridine buffer. [l] In 10% acetonitrile–water, μ = 0.5; all solutions contain 0.03 M imidazole buffer. [m] One point at $\mu > 0.5$.

celerate the departure both of the amine and the alcohol component of the intermediate, to relative extents which are pH dependent.

The complex dependences of product yield on pH (at zero buffer concentration) were qualitatively interpreted in terms of the participation of cationic, neutral, and anionic intermediates.[1a] With the *N*-aryl formimidates I–III and the iminolactone V, it was not possible to evaluate quantitatively the contribution of each species of the intermediate to product formation, owing to the severe overlap of the pH regions where transitions between cationic and neutral, or neutral and anionic, species took place. For that reason, the quantitative treatment previously applied to the effect of buffers on the products of hydrolysis of imidate[4,8] and thioimidate[9,14] esters cannot be used to rigorously identify the reactive ionic species of buffer and intermediate. Insofar as possible, we attempt to suggest reaction mechanisms at least qualitatively consistent with the novel observations made in this study. The

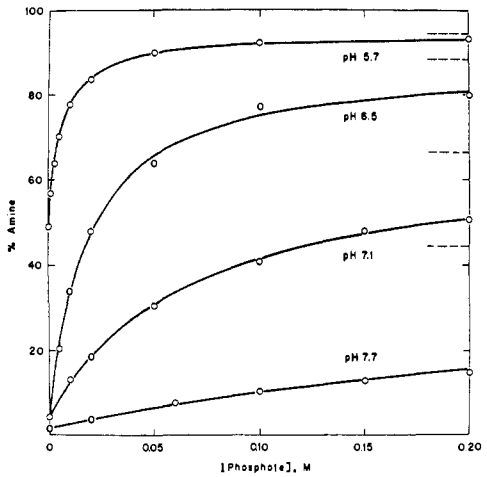
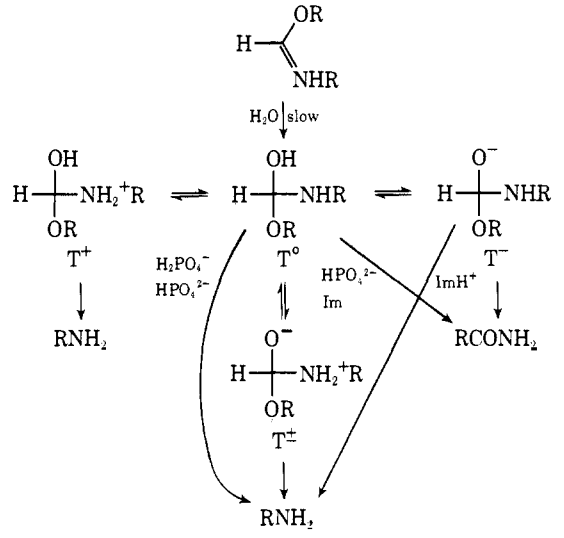


Figure 4. Effect of phosphate buffer on the yield of *p*-nitroaniline obtained on hydrolysis of III. Curves are calculated from eq 1, using the constants given in Table III, and the dashed lines indicate the calculated maximum yields of amine reached at high buffer concentration for each pH value.

formimidates I–III and the iminolactone V will be considered as a group, while the dinitroimidate IV will be discussed separately.

The proposed mechanism of Scheme I appears to

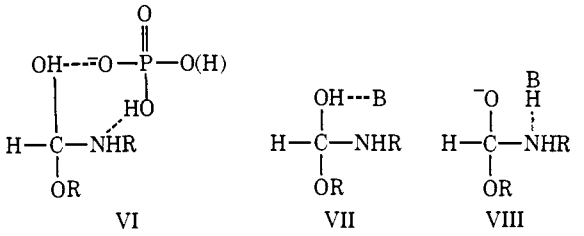
Scheme I



account reasonably for the principal phenomena in the reactions of imidates I–III and V with buffer species. Its major features are: in the absence of general acids or bases, imidate hydrolysis proceeds through the rate-determining formation of tetrahedral intermediates which decompose principally to amine *via* cationic (T^+) and zwitterionic ($T^\pm$) forms and to amide *via* an anionic species (T^-). Monofunctional buffers (*e.g.*, imidazole) catalyze the expulsion of amine by protonation of T^- on nitrogen and may also promote the decomposition of T^0 by proton abstraction and expulsion of alkoxide ion, yielding amide. Bifunctional catalysts ($H_2PO_4^-$, HPO_4^{2-}, and CH_3COOH) efficiently convert the neutral intermediate T^0 to amine but may also act as monofunctional bases to expel alkoxide by proton abstraction.[16]

(16) (a) It has been proposed[16b] that, in many instances, highly reactive intermediates of the type considered here may not be at equilibrium with respect to proton transport processes. The interactions depicted in Scheme I and in formulas VII–XI are not meant to imply that the cleavage of bonds to carbon is necessarily concerted with the proton transfer processes illustrated. Our primary aim is to suggest the nature of the ionic species of catalyst and intermediates which may be involved in the overall process of product formation. It may very well be that diffusion-controlled proton transport reactions are kinetically important in these reactions. In the absence of pertinent data, we have chosen to not explicitly consider this aspect of the reaction mechanism. (b) W. P. Jencks, *Chem. Rev.*, 72, 705 (1972).

The following observations are in agreement with the proposed scheme. (a) The much greater ability of phosphate (pK_a = 6.77 for $H_2PO_4^-$) than of imidazole (pK_a = 7.0) to expel amine from the tetrahedral intermediate indicates that, with I–III, phosphate exhibits the special bifunctional properties earlier found with iminolactones and acyclic imidate esters[4,7,8] (VI). As pH



increases, the dependence of log K_{app} on pH approaches a slope of 1, as reported with other compounds;[4,8] the decreasing effectiveness of the catalyst (in terms of total buffer concentration) may be explained simply by the decreasing concentration of T^0, since at high pH the catalyst exists completely as the reactive HPO_4^{2-} species.

(b) With I–III, the sensitivity of phosphate catalysis to electron withdrawal in the amine was assessed by comparing K_{app} for phosphate at the pH of the midpoint of the "sigmoid" curves relating amine yields to pH[1a] These were pH 6.5, 6.1, and 5.7 for I, II, and III, respectively. The decreasing effectiveness of phosphate as a bifunctional catalyst in the series I–III (see experiments 1, 14, and 21 in Table III) may reflect the decreasing importance of product formation *via* T^0 (or $T^\pm$) in competition with pathways involving T^+ and T^-, as the decreasing basicity of the amine reduces the proportion of the zwitterionic intermediate $T^\pm$. Also, electron withdrawal in the amine may reduce the intrinsic ability of $H_2PO_4^-$ and HPO_4^{2-} to act in a bifunctional sense.

(c) The decreasing maximum yields of amine (Figure 4) suggest the availability of a phosphate catalyzed pathway to amide formation. The sigmoid dependence of maximum yield on pH (Figure 5) requires that the stoichiometric composition of the transition state leading to amide be different by one proton from that of the transition state for amine expulsion. A mechanism involving the interaction of HPO_4^{2-} with the neutral intermediate T^0 (VII) is consistent with these results. Note that there must also exist a pathway to amine formation through a transition state equivalent to (T^0 + HPO_4^{2-}) because it appears that amine production does not fall to zero at high pH but approaches a constant value of about 25%. This pathway might be represented by the bifunctional mechanism VI (using HPO_4^{2-}) or the kinetically indistinguishable monofunctional interaction of $H_2PO_4^-$ with the anionic intermediate T^- (VIII).

(d) The effects of monofunctional catalysts such as imidazole on product formation may be explained

simply by assuming that the relative importances of transition states VII and VIII vary with the nature of the substrate. With relatively basic imidates (*e.g.*, I), amine expulsion (through VIII) is somewhat more important than alkoxide expulsion (through VII), so that amine yield increases with imidazole concentration (Table III). Since both pathways to product involve transition states of the same composition, maximum amine yield should be independent of pH. Also, since the mechanism involves the conjugate acid of the catalyst and the conjugate base of the intermediate (or the reverse), there might be seen an optimum pH for imidazole catalysis (*i.e.*, a minimum in the dependence of K_{app} on pH), which seems to be the case with I (Table III, experiments 6–10). As the basicity of the amine portion of the imidate is reduced by *m*-nitration to II, paths VII and VIII become equally important; imidazole does not affect product yield and it is not possible to calculate the relative efficiencies of imidazole and water as general catalysts for expulsion of amine or alcohol by this type of experiment (*i.e.*, K_{app} is indeterminate). With the least basic substrate III, pathway VII predominates and imidazole catalyzes mainly amide formation. The fact that K_{app} *decreases* with increasing pH (Table III, experiments 28–30) suggests that the maximum effectiveness of imidazole in this system lies at somewhat higher pH than yet reached.[17]

When expressed in terms of the reaction of amine cation with tetrahedral intermediate (presumably T^-), the relative catalytic efficiency of monofunctional amines shows a fairly steep dependence on the pK_a of the amine (Table II). Although the available data are much too limited for conclusive interpretation, the possibility should certainly be considered that the proposed interaction of amine cation with the anionic intermediate T^- (VIII) in fact consists of a stepwise process including first a diffusion-controlled protonation of nitrogen followed by rapid decomposition of the zwitterionic species $T^{\pm}$ to aniline and ester.[9,10,16b,18]

The observation that acetate buffer is an effective catalyst for amine formation from the *p*-nitroimidate III suggests that acetic acid interacts bifunctionally with T^0, as proposed earlier.[8] It is unlikely that the relatively high acidity of acetic acid results in amine expulsion *via* VIII (*i.e.*, by a simple monofunctional pathway), since the nearly as acidic pyridine catalyzes mainly amide formation from III (Table III, experiments 32 and 33).

At zero buffer concentration, the yield of 2,4-dinitroaniline produced on hydrolysis of IV varies with pH as an inverse bell-shaped curve, with a minimum

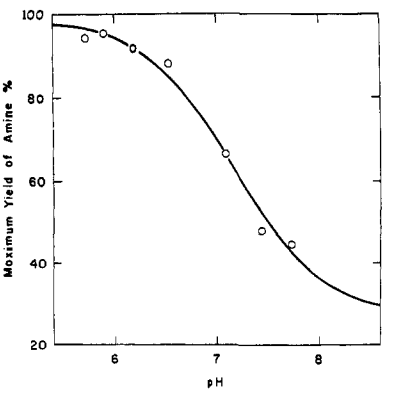
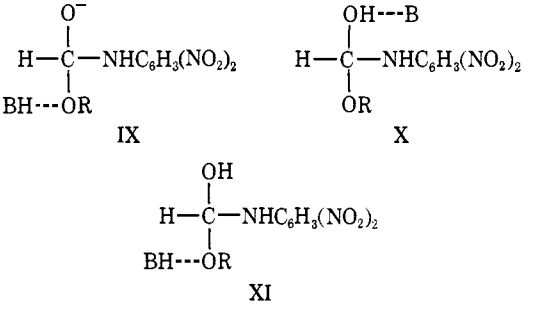


Figure 5. pH dependence of the maximum yields of *p*-nitroaniline obtainable on hydrolysis of III in the presence of phosphate buffer. Line is calculated titration curve of monovalent acid of $pK' = 7.16$, with asymptotes at 98.9 and 27.1%, at low and high pH, respectively.

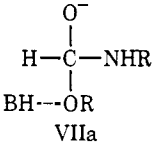
at pH 4.4.[1a] Although the reasons for this unusual pH dependence are not yet understood, it appears that the anionic intermediate T^- expels preferentially the relatively good leaving group 2,4-dinitroaniline anion (whose conjugate acid has $pK_a = 15.0$)[19] while a neutral intermediate breaks down equally well to dinitroaniline and 2,4-dinitroformanilide.

The observation that all buffers tested (at pH ≥ 5) decrease the yield of dinitroaniline suggests that the very low basicity of the nitrogen center (pK_a of 2,4-



dinitroanilinium ion is -4.5)[20] allows protonation at oxygen and departure of ethanol (IX) to compete favorable with expulsion of amine. If this explanation is correct, it is not clear why the asymptotic yields of amine increase with increasing pH (Table III, experiments 34–36 and 38–39). General-base-catalyzed breakdown of T^0 (X) would be expected to enhance expulsion of amine, by analogy to the known effect of hydroxide ion (*i.e.*, T^- gives mostly amine). On the other hand, the relative importance of transition states IX and X is pH independent, since they have the same stoichiometry. It might be suggested that the pathway leading to amide is best accounted for by the general-acid-catalyzed breakdown of T^0 (XI); competition between pathways X and XI would clearly depend on pH. It is known, however, that at low pH, amine yield *increases*, *i.e.*, that hydronium ion catalyzes the conversion of T^0 to amine, and it is not obvious why general acids should favor amide formation. We are not able at present to give a satisfactory general explanation of the effects of buffers in the pathways of breakdown of IV. In any event, phosphate and acetate buffers exhibit no special

(17) In the preceding discussion, we have selected certain transition state structures to account for the experimental observations. Clearly, a number of other kinetically indistinguishable transition states can be envisaged and are not excluded by the data. For instance, structure VIIa is kinetically equivalent to VII and leads to the same hydrolysis



VIIa

products. We have attempted to choose plausible transition state structures, but others cannot be ruled out at present.

(18) (a) R. E. Barnett and W. P. Jencks, *J. Amer. Chem. Soc.*, **91**, 2358 (1969); (b) C. Cerjan and R. E. Barnett, *J. Phys. Chem.*, **76**, 1192 (1972).

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(20) C. H. Rochester, "Acidity Functions," Academic Press, New York, N. Y., 1970, p 25.

ability to enhance the expulsion of either dinitroaniline or ethanol in the hydrolysis of IV.

If the reaction of alcohol and water with nitro-substituted anilides proceeds *via* the formation of tetrahedral intermediates similar to those presumed to occur in the hydrolyses of II–IV, it may be expected that the mechanism of general acid–base catalysis of these reactions will depend both on the nature of the substrate and of the catalyst. For example, it would be anticipated that phosphate and acetate buffers would catalyze the hydrolysis of *p*-nitroformanilide mainly in the pH region of rate-determining breakdown of intermediates. Amine buffers, on the other hand, should exert their catalytic influence mainly in pH regions of rate-determining formation of intermediates, since these monofunctional catalysts appear best capable of increasing the rate of expulsion of alcohol from the tetrahedral intermediate (and hence the rate of addition of alcohol or water to the anilide carbonyl group).

Experimental Section

The preparation or purification of the imidate esters and buffers has been described.[1a]

The hydrolysis of the formimidates I–IV was carried out in 2% CH_3CN–H_2O (v/v), at 30°, and the ionic strength was 0.5 (maintained with added KCl) in all cases except a few reactions at high phosphate buffer concentrations, which are noted in Table III. With the iminolactone V, the solvent was 10% CH_3CN–H_2O. All reactions were allowed to proceed to completion (6–10 half-lives), using the kinetic data obtained in an earlier study.[1a] Reactions were initiated by the addition of 0.1 ml of an acetonitrile solution of the substrate to 5 ml of aqueous buffer solution, previously equilibrated at 30°, followed by vigorous mixing on a Vortex shaker. Final concentrations of imidate esters were generally 0.5–1.5 × 10^{-4} *M*, except for the *m*-nitroformimidate II, where substrate concentration was about 10^{-3} *M*, owing to the small extinction coefficients of the products at the wavelength of measurement.

Analysis for products was carried out by a diazotization assay for aniline (with I) or by direct spectrophotometric examination of reaction mixtures (with II–V), using described procedures.[1a] The identity of the reaction products was confirmed in several cases by comparison of the complete ultraviolet spectra of reaction mixtures to those of synthetic mixtures of the aniline and corresponding formanilide, at the concentration determined by analysis.[1a] Owing to the resemblance of the ultraviolet spectra of 2,4-dinitroaniline (λ_{max} 347 mμ (ε 14,000), 263 (9000), in 1% acetonitrile–water) to that of 2,4-dinitrophenolate ion (λ_{max} 359 mμ (ε 15,000), 256 (7900)),[21] complete spectra were determined of the hydrolysis products of IV under the following conditions: acetate buffer, pH 4.95, 0.01 and 0.50 *M*; phosphate buffer, pH 5.95, 0.01 and 0.1 *M*. The yields of 2,4-dinitroaniline were, respectively, 64.1, 22.3, 79.3, and 60.9%. In all cases, the spectra were identical in the wavelength region 220–450 mμ to those of the corresponding mixtures of 2,4-dinitroaniline and 2,4-dinitroformanilide. Also, no change in spectrum occurred on acidification of the reaction mixture to pH 1; the presence of significant quantities of 2,4-dinitrophenolate ion would have led to a large hypsochromic shift on acidification to 2,4-dinitrophenol (pK_a *ca.* 4, λ_{max} 260 mμ (ε 12,700), shoulder at *ca.* 290 mμ).

(21) *Cf.* D. V. Parke, *Biochem. J.*, **78**, 262 (1961).

Structure of the Peptide Antibiotic Amphomycin[1]

Miklos Bodanszky,* Gerald F. Sigler, and Agnes Bodanszky

Contribution from the Department of Chemistry, Case Western Reserve University, Cleveland, Ohio 44106. *Received October 6, 1972*

Abstract: The structure of amphomycin, elucidated mainly by selective acid degradation, is represented in Figure 1.

Elucidation of the structure of amphomycin, an antibiotic useful in medicine, was suggested to us by Professor David Perlman (of the University of Wisconsin). It is the first described[2] member of a series of closely related peptide antibiotics. Of these, two alternative structures were tentatively proposed[3] for glumamycin,[4–7] but only the amino acid and fatty acid constituents are known in the cases of zaomycin,[8] crystallomycin,[9] aspartocin,[10–12] laspartomycin,[13] and tsushimycin.[14] The structural studies described in this paper point to the identity of glumamycin with amphomycin and also indicate that aspartocin and tsushimycin are different from amphomycin only in respect to the fatty acid constituent, but not in the peptide part of their molecules.

Fatty Acid Constituents and Heterogeneity. Countercurrent distribution of the amphoteric form of amphomycin,[2] in a system of 1-butanol–pyridine–acetic acid–water (4:2:1:7), resulted in a distribution curve that coincided with the curve calculated for the experimentally found distribution coefficient (k = 3.0).[15] Samples taken from different parts of the distribution band were hydrolyzed and analyzed for amino acids; the same ratios of amino acids were found in all of them.

(1) This paper is dedicated to Professor Theodor Wieland to honor him on his 60th birthday.

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