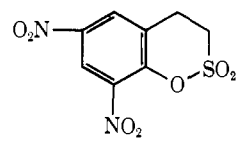
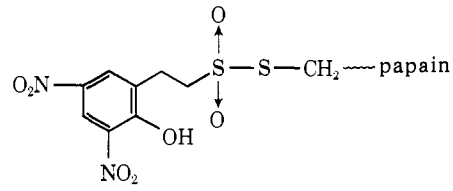
been postulated that an enzyme-bound imidazolium cation interacts with the carboxylate group of the reagents,[20,26] thereby orienting them for proper attack by the sulfhydryl function. If different factors are responsible for the binding of I than that of the α-halo acids, then the higher pK_2 value observed for the reaction of papain with I[36] may represent simply the ionization of the active site sulfhydryl group while, as suggested by others, the pK_2 values seen with the α-halo acids may reflect the dissociation of both the sulfhydryl and imidazole groups.

The pH dependencies for the ionization of the nitrophenol chromophores in papain alkylated by I and by VI are sigmoidal, demonstrating that the ionization of these groups is not detectably affected by that of an enzymatic ionizing group having a comparable pK_a value. This result differs from that obtained with papain sulfonylated by the reactive aromatic six-membered sultone β-(2-hydroxy-3,5-dinitrophenyl)-ethanesulfonic acid sultone (VIII) to give the enzymatic thiolsulfonate species IX.[37] Spectrophotometric titration of the dinitrophenol chromophore present in the thiolsulfonate species IX which is considerably more acidic than the nitrophenol functions present in papain alkylated by I and by VI indicates that the



VIII



IX

ionization of the dinitrophenolic hydroxyl is significantly perturbed by that of a group of similar pK_a at the active site of the enzyme. However, the identity of the perturbing enzyme-bound group has not been established yet.

Finally, a possible explanation for our observations that the nitrophenol groups in papain modified by I and by VI have significantly higher pK_a values than those in the reagents themselves is that the active site has a less polar environment than the aqueous solution. In accord with this interpretation, X-ray studies of crystalline papain indicate that the active sulfhydryl group is located in a nonpolar cleft.[38]

(36) For instance, the pK_2 value for the reaction of papain with L(−)-α-iodopropionic acid at 25.0° is 7.8[26] and with chloroacetic acid at 30.5° it is 8.1.[20]

(37) P. Campbell and E. T. Kaiser, *J. Amer. Chem. Soc.*, **95**, 3735 (1973).

(38) J. Drenth, J. Jansonius, R. Koekoek, and B. Wolthers, "The Enzymes," Vol. 3, 3rd ed, P. D. Boyer, Ed., Academic Press, New York, N. Y., 1971, p 484.

Synthesis of Saturated Isoimides. Reactions of *N*-Phenyl-2,2-dimethylsuccinisoimide with Aqueous Buffer Solutions

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Abstract: The synthesis of *N*-phenyl-2,2-dimethylsuccinisoimide, *N*-(*p*-anisyl)-2,2-dimethylsuccinisoimide, *N*-*n*-butyl-2,2-dimethylsuccinisoimide, *N*-phenyl-*endo*-*cis*-bicyclohept-5-ene-2,3-carboxisoimide, and *N*-(*p*-anisyl)-*endo*-*cis*-bicyclo[2.1.1]hept-5-ene-2,3-carboxisoimide by the dehydration of the corresponding amide acids with *N*,*N*′-dicyclohexylcarbodiimide or ethyl chloroformate–triethylamine reagents is reported. A detailed study of the kinetics of the reactions of *N*-phenyl-2,2-dimethylsuccinisoimide in aqueous buffers demonstrated that hydrolysis to the amide acid follows the rate law $k_{obsd} = 2 \times 10^{-4}\ sec^{-1} + 56\ M^{-1}\ sec^{-1}\ a_{H^+} + 510\ M^{-1}\ sec^{-1}\ a_{OH^-}$. The disappearance of isoimide is also catalyzed by the basic form of the buffers, carbonate, *N*-methylimidazole, acetate, and Dabco, as well as by acetic acid. In the presence of phosphate and tris(hydroxymethyl)aminomethane buffers, the buildup of intermediates was observed. Extensive rearrangement to the corresponding imide generally occurs in the presence of buffers. Saturated isoimides are more reactive than unsaturated isoimides; failure to isolate the former compounds by the direct dehydration of the corresponding amide acids can be attributed to their reactivity rather than to their failure to form.

Phthalisoimides were first synthesized in 1893[2] and the synthesis and chemical behavior of both phthalisoimides and malisoimides have received a considerable amount of more recent attention.[3] Only two reports of the isolation of cyclic isoimides derived

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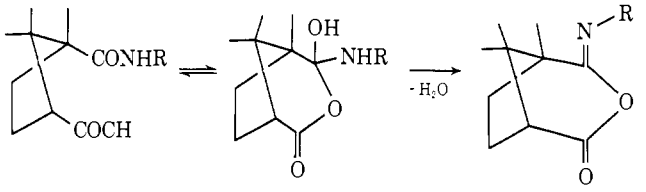
(2) S. Hoogewerff and W. A. van Dorp, *Recl. Trav. Chim. Pays-Bas*, **12**, 12 (1893).

(3) (a) See 3b and 3c and references contained therein: (b) M. L. Ernst and G. L. Schmir, *J. Amer. Chem. Soc.*, **88**, 5001 (1966); (c) C. K. Sauers, C. L. Gould, and E. S. Ioannou, *ibid.*, **94**, 8156 (1972).

from saturated diacids have been made;[4a,5] of these, only the first, the synthesis of camphorisoimide by the dehydration of α- and β-camphoramic acids, involved the direct dehydration of a saturated amic acid to the isoimide.[4]

It has been suggested[5] that the successful syntheses of camphorisoimides (in contrast to the unsuccessful syntheses of succinisoimides and glutarisoimides) derive from the strong driving force for ring closure inherent in the highly substituted camphoramic acids. Carboxyl-assisted hydrolysis of the amide group



of maleamic acids is thought to proceed through similar ring-closed intermediates; the reaction rates have been shown to be very sensitive to alkyl substitution on the amic acid.[6] These results as well as others[7] on the effect of substitution on the rates of ring-closure reactions offer support for this suggestion.

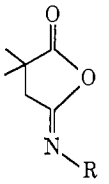
An alternative explanation for the failure to obtain isoimides from succinamic and glutaramic acids stems from the very ready hydrolysis[3b] of cyclic isoimides and the facile rearrangements to imides[3] which have been observed for maleisoimides and phthalisoimides. Thus various dehydrating agents may convert saturated amic acids to isoimides which are subsequently hydrolyzed or converted to other products during the work-up procedures. The behavior of succinamic acids toward trifluoroacetic anhydride may be interpreted in this way.[4b]

Our interest in this problem stems not only from the desire to gain an answer to the above question but also from the possibility that isoimides may be biochemical intermediates. The hypothesis has been raised that the amide groups of proteins may function as nucleophilic catalysts in biological processes.[3b] Such catalysis would lead to isoimide intermediates in neutral and acidic media where the nucleophilicity of the amide resides on the carbonyl oxygen. Furthermore, the saturated isoimide derived from asparagine may be an unstable chemical intermediate in the recently demonstrated conversion of the neurotoxic principle in common vetch, γ-L-glutamyl-L-β-cyanoalanine, to asparagine.[8] Accordingly, the behavior of model saturated isoimides might be relevant to these systems.

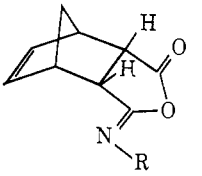
In this paper, we report the results of a study of the synthesis of saturated cyclic isoimides and a detailed study of the kinetics and mechanisms of the reactions of aqueous buffer solutions with *N*-phenyl-2,2-dimethylsuccinisoimide.

Results and Discussion

Synthesis. A variety of saturated isoimides, **1a–c, 2a,** and **2b**, were synthesized from the corresponding amic acids using the previously developed[9] dehydrating reagents, *N,N'*-dicyclohexylcarbodiimide and ethyl chloroformate–triethylamine. The reaction products were more reactive than the maleisoimides and phthalisoimides, but the use of gentle work-up procedures led to the isolation and characterization of five new saturated isoimides. (Although **2a** and **2b** each have

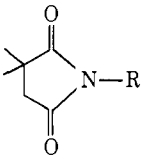


1a, R = phenyl
b, R = *p*-anisyl
c, R = *n*-butyl

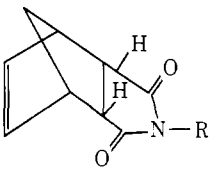


2a, R = phenyl
b, R = *p*-anisyl

a double bond, the ring containing the isoimide group is saturated.) The imides **3a–c**, **4a**, and **4b** were synthesized for comparison purposes and data for these compounds as well as for the isoimides are summarized in Table I.



3a, R = phenyl
b, R = *p*-anisyl
c, R = *n*-butyl



4a, R = phenyl
b, R = *p*-anisyl

The saturated isoimides exhibit the two sharp absorptions in the carbonyl region of the infrared which have been previously used as criteria for the isoimide structure.[10] Occasionally two peaks appear in the carbonyl region of the spectra of the imides as well (see compounds **2a** and **4a** in Table I); the peak above 1800 is strong for isoimides and weak for the imides. The uv spectral differences between isoimides and imides which have been noted for maleisoimides[10] are also characteristic of the saturated isoimides reported here. The most intense absorption for the imides lies below 230 nm while the corresponding *N*-aryl substituted isoimides exhibit absorption maxima from the C=N-aryl chromophore at longer wavelengths.

The nmr spectra are consistent with the assigned structures. Compounds **1a–c** exhibit two peaks each for the *gem*-dimethyl protons and methylene protons. Spectra of mixtures of these compounds with the corresponding imides demonstrated that the "extra" peaks could not be attributed to the presence of imides as impurities. When the isoimide spectra were recorded at elevated temperatures the peaks collapsed to single sharp resonance lines; the original spectra were

(4) (a) S. Hoogewerff and W. A. van Dorp, *Recl. Trav. Chim. Pays-Bas*, **12**, 15, 17 (1893); **14**, 261, 266, 269 (1895); (b) W. R. Roderick and P. L. Bhatia, *J. Org. Chem.*, **28**, 2018 (1963).

(5) E. Hedaya, R. L. Hinman, and S. Theodoropulos, *ibid.*, **31**, 1317 (1966).

(6) (a) A. J. Kirby and P. W. Lancaster, *Biochem. J.*, **117**, 51P (1970); *J. Chem. Soc., Perkin Trans. 2*, 1206 (1972); (b) M. F. Aldersley, A. J. Kirby, and P. W. Lancaster, *J. Chem. Soc., Chem. Commun.*, 570 (1972).

(7) T. C. Bruice and U. K. Pandit, *J. Amer. Chem. Soc.*, **82**, 5858 (1960).

(8) C. Ressler, S. N. Nigam, and Y.-H. Giza, *ibid.*, **91**, 2758, 2766 (1969).

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(10) E. Hedaya, R. L. Hinman, and S. Theodoropulos, *ibid.*, **31**, 1311 (1966).

Table I. Characterization of Imides and Isoimides

Compd	Mp, °C[a]	Ir,[b] cm^{-1}	λ_{max}^{uv}, nm,[c] ϵ	Nmr, $CDCl_3$–TMS,[d] δ	Microanalyses[e] Calcd C	Calcd H	Found C	Found H
1a	66–68	1815, 1700	253, 4180	1.37, 1.42 (6 H); 2.70, 2.96 (2 H); multiplet 7.2 (5 H)	70.91	6.45	70.84	6.76
3a	85–87[f]	1720	253,[h] 380	1.42 (6 H); 2.72 (2 H); 7.43 multiplet (5 H)				
1b	50–53	1810, 1710	261, 7500	1.36, 1.42 (6 H); 2.73, 2.93 (2 H); 3.81 (3 H); multiplet 7.05 (4 H)	66.93	6.48	66.65	6.59
3b	92–92.5	1700	273, 1300	1.39 (6 H); 2.68 (2 H); 3.81 (3 H); quartet 7.13 (4 H)	66.93	6.48	66.84	6.64
1c	bp 73–74 (2 mm) $n^{26}D$ 1.4560	1830, 1740		0.92 m (3 H); 1.38 s (6 H); 1.50 m (4 H); 2.72 m (2 H); 3.42 t (2 H)	65.54	9.35	65.31	9.64
3c	bp 69–69.5 (2.5 mm)	1723		0.92 m (3 H); 1.32 s (6 H); 1.44 m (4 H); 2.55 (2 H); 3.50 t (2 H)	65.54	9.35	65.32	9.59
2a	106–108	1810, 1725	255, 4100	1.67 m (2 H); 3.50 m (4 H); 6.36 (2 H); 7.20 m (5 H)	75.30	5.48	75.44	5.60
4a	142–143[g]	1730, 1810 weak	255,[h] 374	1.68 m (2 H); 3.43 m (4 H); 6.27 (2 H); 7.33 m (5 H)				
2b	73–74.5	1805, 1725	266, 7240	1.63 m (2 H); 3.48 m (4 H); 3.78 s (3 H); 6.34 (2 H); 6.97 m (4 H)	71.36	5.61	71.28 71.20	5.87 5.90
4b	171–172	1700	273, 1400	1.70 m (2 H); 3.44 m (4 H); 3.80 s (3 H); 6.27 (2 H); 7.02 (4 H)	71.36	5.61	71.38	5.77

[a] Uncorrected. [b] Nujol mull or liquid film. [c] In acetonitrile. [d] *Ca.* 10% in $CDCl_3$ with TMS as internal standard. [e] Performed by G. Robertson, Florham Park, N. J. 07932. [f] Lit. mp 86°: "Rodd's Chemistry of Carbon Compounds," Vol. IB, 2nd ed, S. Coffey, Ed., Elsevier, New York, N. Y., 1971, p 975. [g] Lit. mp 144°: I. Heilbron, "Dictionary of Organic Compounds," Vol. I, 4th ed, Oxford University Press, New York, N. Y., 1965, p 395. [h] Shoulder, no maxima above 230 nm.

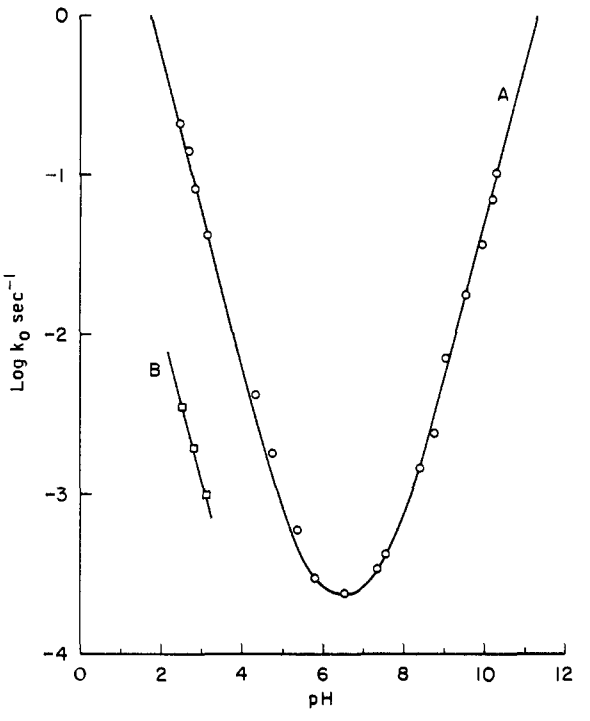


Figure 1. Curve A: pH–rate profile for hydrolysis of *N*-phenyl-2,2-dimethylsuccinisoimide (**1a**) at 25.0° in 10% acetonitrile–water. The solid line is calculated from eq 1 using the constant given in the text. Curve B: partial pH–rate profile for *N*-phenylphthalisoimide. Rate constants are expressed in units of sec^{-1}.

obtained when the samples were recooled. We interpret these results as evidence for syn–anti isomerism about the carbon–nitrogen double bond in these isoimides.[11]

Spectral evidence for the synthesis of *N*-(*p*-anisyl)-succinisoimide was obtained, but ready hydrolysis to the amic acid occurred during work-up procedures. Boyd has isolated the perchlorate salt of this and other succinisoimides from the dehydration of the amic acids with acetic anhydride–perchloric acid mixtures, but was unable to convert the saturated compounds to the free isoimides.[12] *N-tert*-Butyl-2,2-dimethylsuccinisoimide proved surprisingly susceptible to hydrolysis in view of the easy isolation of **1c**; carbon–hydrogen analysis of the *tert*-butyl compounds repeatedly indicated the presence of the amic acid. The isoimides were not stable to preparative gas phase chromatography conditions. Thus a crude preparation of racemic *N*-*n*-butyl-2-methylsuccinisoimide was converted to the corresponding imide and **2b** underwent a reverse Diels–Alder reaction to produce the known *N*-(*p*-anisyl)maleisoimide.[13]

These synthetic studies demonstrate that the major difficulty in achieving syntheses of most saturated cyclic isoimides cannot be assigned to their failure to form, but must be attributed to their ready transformation to other compounds during standard work-up procedures. The kinetic studies which follow confirm this.

Kinetic Studies. The pH–rate profile at 25° in 10% acetonitrile–water for the hydrolysis of *N*-phenyl-2,2-dimethylsuccinisoimide (**1a**) is shown in Figure 1. The points shown are based on the data summarized in Table II. Since all the buffers used increased the rate

(11) Details of syn–anti isomerism for the cyclic isoimide system have not been previously reported in the literature although this phenomenon has been observed for the *N*-arylmaleisoimides: H. Relles and C. K. Sauers, *J. Amer. Chem. Soc.*, in press.
(12) G. V. Boyd, *Chem. Commun.*, 1147 (1969).
(13) C. K. Sauers, *J. Org. Chem.*, **34**, 2275 (1969).

Table II. Kinetic Data for the Reaction of Aqueous Buffer Solutions with Isoimide **1a**[a]

Buffer	Concn range, M	pH	Mole fraction free base	k_1, sec^{-1}	k_2', M^{-1} sec^{-1}
HCl	0.0045	2.422		0.210	
	0.0027	2.620		0.141	
	0.0018	2.800		0.0816	
	0.00090	3.131		0.0421	
Acetate	0.047–0.187	4.32	0.28	0.00420	0.0105
	0.0179–0.179	4.76	0.50	0.0018	0.00845
	0.047–0.188	5.35	0.81	0.000595	0.00506
	0.0248–0.099	5.80	0.91	0.00030	0.00400
N-Methyl-imidazole	0.0451–0.181	6.56	0.25	0.00024	0.0133
	0.051–0.102	7.35	0.55	0.00034	0.0300
	0.044–0.177	7.57	0.75	0.00042	0.0389
Dabco	0.045–0.180	8.40	0.20	0.00145	0.0550
	0.043–0.173	8.76	0.38	0.0024	0.0956
	0.046–0.184	9.02	0.51	0.0071	0.137
Carbonate	0.036–0.180	9.51	0.20	0.018	0.060
	0.016–0.162	9.94	0.35	0.035	0.100
	0.00725–0.0725	10.19	0.51	0.0700	0.195
	0.018–0.180	10.26	0.60	0.102	0.210

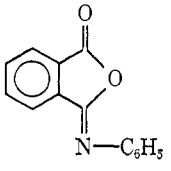
[a] In 10% acetonitrile–water (v/v) at 25.0°; ionic strength = 0.21.

of disappearance of **1a**, the points for the reaction above pH 3.2 were extrapolated to zero buffer concentration. The subsequent carboxylic acid catalysis of amide hydrolysis[14d] did not interfere; the hydrolysis of 2,2-dimethylsuccinanilic acid at 25° has a rate constant of 7.62×10^{-6} sec^{-1} between pH 2.5 and 3.0, where it would be expected to be at a maximum for the pH range 2–12.

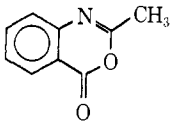
As in the hydrolysis of *N*-phenylphthalisoimide, the data indicate the existence of hydronium ion and hydroxide ion catalysis as well as a small water reaction. Curve A shown in Figure 1 was calculated from eq 1,

$$k_0 = k_{H_2O} + k_H[a_{H^+}] + k_{OH}[a_{OH^-}] \quad (1)$$

with $k_{H_2O} = 2 \pm 1 \times 10^{-4}$ sec^{-1}, $k_H = 56 \pm 3$ M^{-1} sec^{-1}, and $k_{OH} = 510 \pm 90$ M^{-1} sec^{-1}. These values may be compared with values for *N*-phenylphthalisoimide (**5**) at 30° in 10% acetonitrile–water where



5



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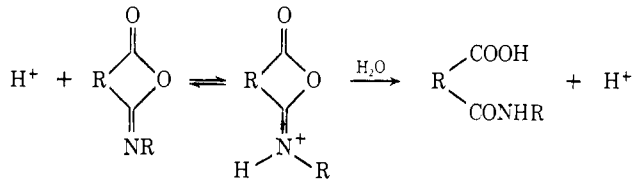
$k_{H_2O} = 1.53 \times 10^{-4}$ sec^{-1}, $k_H = 1.55$ M^{-1} sec^{-1}, and $k_{OH} = 500$ M^{-1} sec^{-1}.[3b] For 2-methyl-3,1-benzoxazin-4-one (**6**), $k_H = 31.6$ and $k_{OH} = 56.2$ M^{-1} sec^{-1}.[15] Curve B was drawn from data on the hydrolysis of *N*-phenylphthalisoimide at 25° in 10% acetonitrile–water ($\mu = 0.21$); k_H under these conditions is about 1.1 M^{-1} sec^{-1}. Thus *N*-phenyl-2,2-dimethylsuccinisoimide is 50 times more reactive than *N*-phenylphthalisoimide toward hydronium ion catalysis. This difference can be largely attributed to the expected differences in basicity of the two compounds since the mechanism of specific acid catalysis in a similar system has been shown to involve water attack on the immonium ion center.[16] The oxazinone **6** is also attacked at the immonium center;[15] since its basicity would be expected to lie intermediate between the basicities of **1a** and **5**, the intermediate value of k_H can also be explained on that basis.



The catalytic constants for hydroxide ion attack and for the water reaction for *N*-phenylphthalisoimide at 30° and for the saturated isoimide **1a** at 25° are of the same order of magnitude. The saturated compound appears to be only slightly more reactive than the isoimide derived from the aromatic diacid. Hydroxide attack would be expected to occur at the carbonyl carbon of **1a**, as has been shown to be the case for *N*-*n*-butylmaleisoimide[17] and *N*-phenylmaleisoimide.[16] The rate of such attack would be expected to be retarded by the *gem*-dimethyl substitution next to the carbonyl group and therefore unsubstituted succinisoimides would be predicted to be even more reactive than compound **1a**. This greater reactivity contributes to the difficulties encountered in the synthesis and isolation of these compounds.

The buffer catalysis observed can be described by eq 2 where k_0 is equal to the water reaction at a given

$$k_{obsd} = k_0 + k_2'[B_T] \quad (2)$$

pH (defined in eq 1) and k_2' is the apparent second-order rate constant for catalysis by the total buffer concentration $[B_T]$. For the buffers, *N*-methylimidazole, Dabco, and carbonate, k_2' increases with increasing mole fraction of buffer in the conjugate base form (see Table II and Figure 2). The values of k_2' for carbonate exhibit more scatter than do the other buffers. Only a small percentage of the reaction proceeds through buffer catalysis in the pH region of carbonate–bicarbonate buffers. General acid catalysis by acetic acid is of greater importance than catalysis by acetate ion.

The analysis of the individual rate constants for the catalysis by the acid and base forms of the buffer was carried out according to the method developed for the *N*-phenylphthalisoimide reactions with aqueous buffers.[3b] Thus the base form of the buffer (B) may react either with the neutral isoimide (I) or with its conjugate acid (IH) (eq 3). Let $[I_T] = [I] + [IH]$ and

$$k_2'[B_T] = k_1[IH][B] + k_2[I][B] \quad (3)$$

$[B_T] = [B] + [BH]$ with K_1 = the acid dissociation constant of the protonated isoimide and K_2 = the acid dissociation constant of the conjugate acid form of the buffer. Equations 4 and 5 may be derived from these

$$k_2'[B_T] = k_1K_2/K_1[BH] + k_2[B] \quad (4)$$

$$k_2' = k_1K_2/K_1 + [B]/[B_T](k_2 - k_1K_2K_1) \quad (5)$$

(14) (a) M. L. Bender, Y. Chow, and F. Chloupek, *J. Amer. Chem. Soc.*, **80**, 5380 (1958); (b) G. Dahlgen and N. L. Simmerman, *J. Phys. Chem.*, **69**, 3626 (1965); (c) J. Brown, S. C. K. Su, and J. A. Shafer, *J. Amer. Chem. Soc.*, **88**, 4468 (1966); (d) T. Higuchi, L. Eberson, and J. McKae, *ibid.*, **89**, 3001 (1967).

(15) A. Williams and G. Salvadori, *J. Chem. Soc. B*, 1105 (1971).

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(17) R. Paul and A. S. Kende, *J. Amer. Chem. Soc.*, **86**, 4162 (1964).

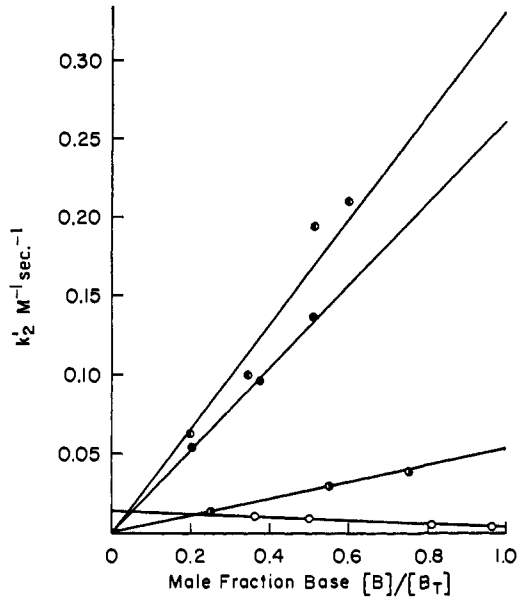


Figure 2. Dependence of apparent second-order rate constant for acetate, *N*-methylimidazole, Dabco, and carbonate on the mole fraction of buffer in the basic form: (○) acetate; (◑) *N*-methylimidazole; (●) Dabco; (◐) carbonate.

definitions and eq 3. This derivation depends on the assumption that in the pH range of interest the isoimide exists almost entirely as the neutral species. This assumption is reasonable in view of the linearity of the pH–rate profile in the lower pH regions which indicates that the pK_a of protonated **1a** lies below 2. The pK_a of **1a** can be estimated to be below 1.5 from the effect of oxygen substituents on the pK_a's of *N*-methylacetimidates and the pK_a of *N*-phenyliminobutyrolactone.[18]

Values for k_2 derived from eq 5 and the plots shown in Figure 2 are listed in Table III along with second-order rate constants for nucleophilic catalysis for *N*-phenylphthalisoimide and *p*-nitrophenyl acetate. The value for k_1K_2/K_1 for acetic acid at 25° is 0.013 which is an order of magnitude larger than the value of 0.0014 for acetic acid catalysis of the disappearance of **5a** at 30°.[3b] This difference probably reflects the expected greater basicity (and thus smaller K_1) of isoimide **1a** compared to **5** and parallels the similar differences observed in hydronium ion catalysis of these two compounds, *vide supra*. Two kinetically equivalent mechanisms are possible for the interpretation of the acetic acid catalysis. In addition to acetate attack on the protonated isoimide, which is described in eq 2, the kinetic data would be equally well explained by general acid catalyzed water attack on the carbon–nitrogen double bond of **1a**. Thus if the first term in eq 2 were replaced by k_1'[I][HB], it

(18) (a) Use of Taft's polar substituent constants, σ^*, for the substituents on oxygen of the *N*-methylacetimidates studied in ref 18b yields a ρ^* value of +2.0 for the acid ionization constants of these protonated acetimidates. Use of this ρ^* value combined with the pK_a of 5.06 determined for *N*-phenyliminobutyrolactone[18c] gives a value of 1.5 for the pK_a of compound **1a**. This value would be expected to be an upper limit because it does not take into account the small amount of stabilization of the unprotonated **1a** through delocalization of the charge of the polarized carbonyl group to the ring oxygen. (b) T. Pletcher, S. Koehler, and E. H. Cordes, *J. Amer. Chem. Soc.*, **90**, 7072 (1968); (c) G. L. Schmir and B. A. Cunningham, *ibid.*, **87**, 5692 (1965); (d) for a further discussion of amide and acetimidate protonation equilibria, see A. Fersht, *ibid.*, **93**, 3504 (1971).

Table III. Second-Order Rate Constants for the Reactions of Conjugate Bases of Buffers with Neutral Isoimide **1a**, *N*-Phenylphthalisoimide, and *p*-Nitrophenyl Acetate

Buffer	pK^a	k_2,[b] M^{-1} sec^{-1}	k_2,[c] M^{-1} sec^{-1}	k_2,[d] M^{-1} sec^{-1}
H_2O	−1.7	4×10^{-6}	3.1×10^{-6}	1×10^{-6}
Acetate	4.71	0.0032	0.0036	8.5×10^{-6}
N-Methylimidazole	7.14	0.053		0.317[e]
Dabco	8.98	0.26		0.0323
Carbonate	10.21	0.34	1.6	0.018
Hydroxide	15.7	510	500	14.8

[a] pK values for the dissociation of the conjugate acid of the buffer determined in this study at 25.0°, μ = 0.21 in 10% acetonitrile–water. [b] In 10% acetonitrile–water at 25.0°, μ = 0.21. [c] In 10% acetonitrile–water at 30.0°, μ = 0.45.[2] [d] In water at 25.0°; from W. P. Jencks and J. Carriuolo, *J. Amer. Chem. Soc.*, **82**, 1778 (1960), except as noted. [e] In 5% dioxane in water: M. L. Bender and B. W. Turnquest, *ibid.*, **79**, 1656 (1957).

can be shown that $k_1 = k_1'K_1/K_2$. These two mechanisms can be differentiated by product studies since general acid catalysis of water attack can only lead to amic acid as the product, whereas nucleophilic attack of acetate on the protonated isoimide can lead to some imide as product. The latter pathway has been shown to describe the course of formic acid catalysis of *N*-phenylphthalisoimide.[3b]

Product Studies. Table IV summarizes the results

Table IV. Product Studies of the Reactions of Isoimide **1a** with Aqueous Buffer Solutions[a]

Buffer	pH	Total buffer concentration	% amic acid[b]	% imide[b]	% base catalysis	% acid catalysis
Acetate	5.80	0.099	63	37	40	15
	5.80	0.050	73	27	29	9
	4.32	0.187	82	18	4	32
N-Methylimidazole	6.64	0.184	22	78	87	0
	7.54	0.18	19	81	94	0
	7.54	0.094	22	78	88	0
Dabco	8.40	0.09	98	<2	77	0
	8.40	0.045	100	0	63	0
Carbonate	10.70	0.190	100	0	<1	0
	10.17	0.0725	95	5	23	0
	9.94	0.162	84	16	28	0
	9.51	0.180	80	20	30	0
Tris	8.87	0.72	12	88		
Phosphate	7.96	0.24	38	62		
	8.16	0.78	37	63		

[a] In 10% acetonitrile; μ = 0.21 except for Tris and phosphate and pH 10.70 carbonate experiments. [b] Determined from infinity spectra from kinetic runs. For details see Experimental Section.

of product studies carried out by uv analysis of completed kinetic runs. The yield of imide from acetate buffer at pH 4.32 is higher than the percentage of the reaction which proceeds through acetate catalysis; since the water reaction can only lead to amic acid as product, some imide must be produced by acetic acid catalysis of isoimide disappearance. The data in Table IV also show that imide is produced by nucleophilic catalysis by acetate, *N*-methylimidazole, and carbonate. The latter two buffers do not exhibit catalysis by the conjugate acid form. These rearrangements of isoimides to imides parallel

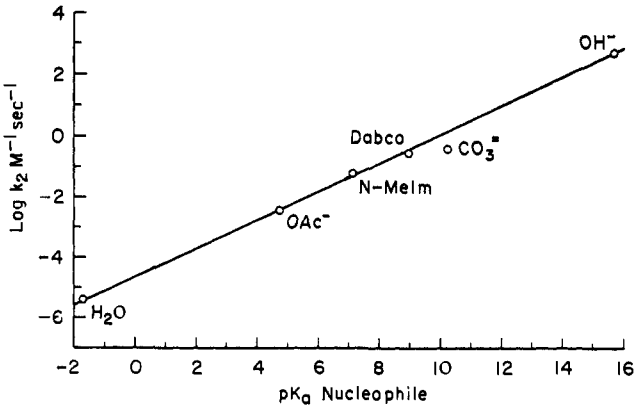
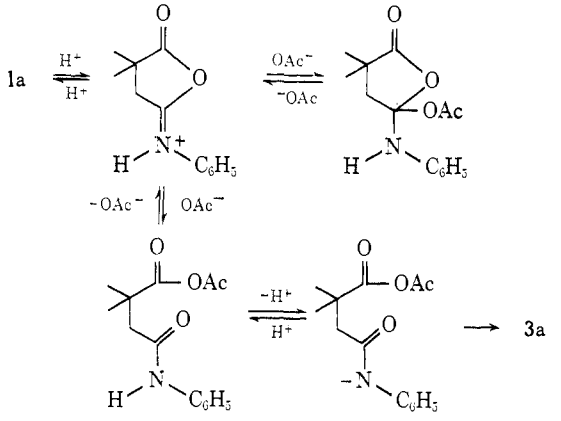


Figure 3. Brønsted plot for catalysis by the conjugate base forms of the buffers, $\beta = 0.47$.

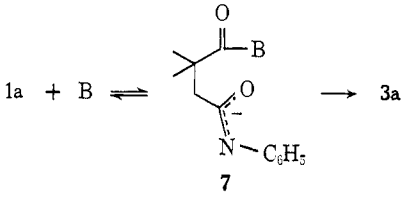
those previously reported,[3b,3c,13] and can be understood in terms of the mechanisms shown in Scheme I.[3b]

Scheme I

Acetic Acid Catalysis



Nucleophilic Catalysis



7

The intermediate formed in nucleophilic catalysis can be diverted from imide formation by hydrolysis at the original carbonyl group to give the amic acid. Since the imide yields are less than the percentage of the reaction proceeding through catalysis by the conjugate base form of the buffers, this process may occur. Alternatively, the catalysis may proceed partially by the nucleophilic pathway and partially by general base catalysis of water attack at the carbonyl group.

Dabco catalysis must occur largely by the latter pathway since little if any imide is formed. Steric hindrance to the attack of this bulky amine on the already crowded carbonyl group apparently prohibits nucleophilic catalysis in this case. Williams and Salvadori found general base catalysis of water attack for compound **6** with imidazole, phosphate, and borate buffers and noted that possible intermediates formed by nucleophilic attack at the carbonyl group could readily react with the oxygen of the neighboring amide group to give starting material.

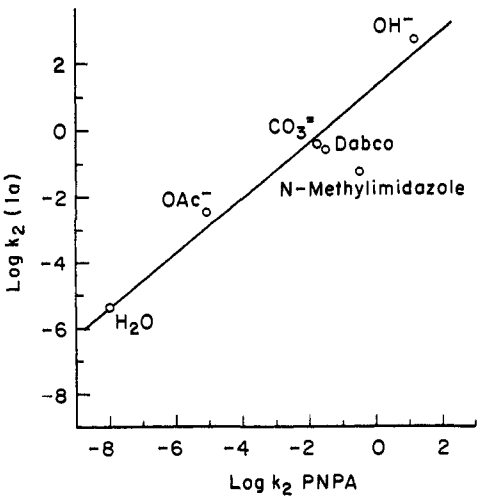


Figure 4. Log k_2 for buffers used in this work *vs.* log k_2 for the reaction of these buffers with *p*-nitrophenyl acetate.

Examination of the data in Table III reveals a relationship between k_2 values and the pK_a's for the conjugate acids of the corresponding nucleophiles. Such a correlation was not found for all the buffers studied by Ernst and Schmir in their work on **5**;[3b] however, those buffers which coincide with the buffers used in the present work do show such a relationship. A Brønsted plot for the k_2 values obtained for **1a** is shown in Figure 3. The β value calculated from these data is 0.47 which is close to that obtained from the limited data for compound **5** listed in Table III. Comparison may also be made with the Brønsted β of 0.67 for compound **6**.[16]

Figure 4 shows a plot of the k_2 values for compound **1a** against k_2 values for *p*-nitrophenyl acetate. Since imide is formed from catalysis by all the buffers used except Dabco, catalysis must occur primarily by the nucleophilic mechanism. The deviation of the point for *N*-methylimidazole is probably related to the greater steric hindrance to attack of **1a** compared to *p*-nitrophenyl acetate. The slope of the plot in Figure 4 is 0.83; for *N*-phenylphthalisoimide (**5**) the corresponding value is 0.87.[3a] Therefore it appears that the more reactive substrates, the isoimides, are slightly less sensitive to the nucleophilicity of the catalyst than is *p*-nitrophenyl acetate. This could be because the transition state occurs earlier in the reaction coordinate for the former compounds.[19]

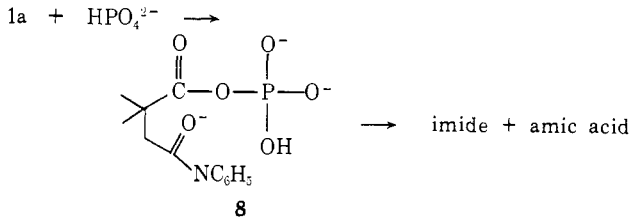
The acetate-catalyzed rearrangement of *N*-arylmaleisoimides to the imides in acetic anhydride is fairly sensitive to substituents in the para position of the aryl group on nitrogen ($\rho = 1.7$).[13] These results indicate that for this reaction in a nonpolar medium, considerable bond breaking to the leaving amide anion has occurred in the transition state. A more detailed structure–reactivity study of the reactions of isoimides with nucleophiles, such as that which has been done on ester alcoholysis and aminolysis reactions,[19] is needed to provide a detailed picture of the transition states of these reactions.

Phosphate and Tris Buffers. Phosphate and Tris buffers each catalyzed the disappearance of isoimide **1a** in a two-stage process. Absorbance *vs.* time data clearly

(19) W. P. Jencks and M. Gilchrist, *ibid.*, **90**, 2622 (1968).

indicated the buildup of an intermediate which then went on to products. Based on the behavior of the nucleophiles discussed above, the most probable explanation of this phenomenon is the formation of the intermediate corresponding to **7** (and its conjugate acid) followed by a slower decomposition to products as illustrated for phosphate in Scheme II. The reac-

Scheme II



8

tion to form imide from **8** would be slow because of electrostatic repulsion between the ionized amide and the doubly charged phosphate moiety. Evidence for the intermediacy of **8** was obtained; repetitive scanning of the reaction mixture showed that the maximum absorbance at the peak of intermediate buildup corresponded to an ϵ of 11,100. This maximum for the intermediate occurred at 242 nm, the same wavelength at which the amic acid exhibits a maximum; the value of ϵ was 95% of that recorded for the amic acid. Species **8** would be expected to have a spectrum almost identical with that of the amic acid. Therefore, spectra of the reaction mixture at intermediate stages of the reaction when the isoimide has almost disappeared and the imide has not yet formed in large amounts should resemble the spectrum of the amic acid. Spectra of alternative intermediates such as those which might be formed from the addition of phosphate across the carbon–nitrogen double bond would differ markedly from that of the amic acid.

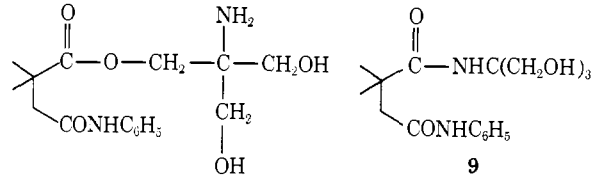
The work that has been done on reactions of acetyl phosphate with water and nucleophiles[20,21] is consistent with the suggested intermediacy of **8**. Thus the hydrolysis of acetyl phosphate dianion occurs at a constant rate independent of phosphate concentration in the pH region of 6–10[20] and has a half-life of about 1200 min.[21] The rate of the decomposition of the intermediate formed from isoimide **1a** at pH 7.96 in 0.25 total phosphate buffer has a half-life of 65 min. This 20-fold increase in rate must arise from the attack of the anion of the neighboring amide group to produce imide. Product studies confirm the latter product.

The hydroxamic acid test confirmed the continued presence of an activated acyl compound during the course of the reaction. The yields of hydroxamic acid gradually dropped to 37% of that expected from the corresponding concentration of isoimide under conditions where about 63% of the reaction proceeded through the intermediate and then gradually rose again. Controls showed that the isoimide gave only 80% of the hydroxamic acid yield given by the corresponding concentration of imide. The yields of the hydroxamic acid from acetyl phosphate have been shown to be less than theoretical (84%)[21] and the steric hindrance of the two methyl groups in **7** might be expected to further decrease the efficiency of this assay.

(20) D. E. Koshland, Jr., *J. Amer. Chem. Soc.*, 73, 4103 (1951); 74, 2286 (1952).

(21) G. DiSabato and W. P. Jencks, *ibid.*, 83, 4393, 4400 (1961).

The behavior of **1a** with Tris buffers showed that the second step of the reaction was catalyzed by the conjugate base of the buffer as well as by hydroxide ion. The second-order rate constant for the latter process was roughly determined to be 800 M^{-1} sec^{-1}. This value is somewhat smaller than the value of 3000 M^{-1} sec^{-1} for the second-order rate constant for hydroxide catalyzed ring closure of methyl *N*-phenylphthalamide, where the neighboring ester and amide groups are held in close proximity.[22] The second-order rate constant for the hydroxide catalyzed formation of carbobenzyloxy-L-aspartylimide from the ester amide, is 3–4 M^{-1} sec^{-1}.[23] Here the free rotation of the starting material and the lower acidity of the primary amide contributes to the slower rate. Thus it seems probable that the intermediate formed from the reaction of Tris with isoimide **1a** is the ester **9** although the amide **10** is not ruled out.



8 **9**

Experimental Secton

Succinamic Acids and Succinimides. The succinamic acids were prepared by the addition of purified amines to the corresponding anhydrides:[14d] 2,2-dimethylsuccinanilic acid, mp 185–187°; *N*-(*p*-anisyl)-2,2-dimethylsuccinamic acid, mp 156–157°; *N*-*n*-butyl-2,2-dimethylsuccinamic acid, mp 129–132°; *endo-cis*-bicyclo-[2.2.1]heptene-2,3-dicarboxylic acid monoanilide, mp 151–152°; *endo-cis*-bicyclo[2.2.1]heptene-2,3-dicarboxylic acid mono-*p*-anisilide, mp 154.5–155°; *N*-*n*-butyl-2-methylsuccinamic acid, mp 68–71°. Melting points agreed with those previously reported in the literature. The imides **3a–c**, **4a**, and **4b** were prepared by dehydration of the corresponding amic acids with acetic anhydride–sodium acetate–acetic acid mixtures.[24] Spectral and analytical data for these compounds are given in Table I.

Saturated Isomides. Except for **1c**, the saturated isoimides were prepared by dehydration of the corresponding amic acids with *N*,*N*′-dicyclohexylcarbodiimide (DCC).[9] For example, 2,2-dimethylsuccinanilic acid, 5.5 g (0.025 mol), was added all at once to an equimolar portion of DCC in 30 ml of dichloromethane. The mixture was stirred 22 hr, then filtered to remove the urea. The filtrate was evaporated under reduced pressure and the residue dissolved in benzene and passed through a column of 100–200 mesh Florisil (deactivated by exposure to room conditions). The resultant benzene eluent was evaporated to a colorless oil which solidified on standing to yield 3.5 g (69%) of isoimide **1a**. Yields using this method were in the range of 65–80%.

During one preparation of compound **2b**, the crude isoimide was injected into the gas chromatograph (inlet temperature 178°, column temperature 143°). The product of the reverse Diels–Alder reaction, *N*-(*p*-anisyl)maleisoimide, was collected and was shown to be identical with an authentic sample[4b,13] of this material.

N-*n*-Butyl-2,2-dimethylsuccinamic acid was dehydrated to the corresponding isoimide by the ethyl chloroformate–triethylamine procedure previously described for the synthesis of maleisoimides.[9] Short path distillation gave a 60% yield of product with ir and nmr spectra consistent with the isoimide structure.

Nuclear magnetic resonance spectra of the isoimides in acetonitrile-d_3, chloroform-*d*, dimethyl-d_6 sulfoxide, and carbon tetrachloride indicated the presence of syn–anti isomerism about the

(22) (a) M. T. Behme and E. H. Cordes, *J. Org. Chem.*, **29**, 1255 (1964); (b) J. A. Shafer and H. Morawetz, *ibid.*, **28**, 1899 (1963).

(23) S. A. Bernhard, A. Berger, J. H. Carter, E. Katchalski, M. Sela, and Y. Shalitin, *J. Amer. Chem. Soc.*, **84**, 2421 (1962).

(24) N. E. Searle, U. S. Patent 2,444,536 (1948); *Chem. Abstr.*, **42**, 7340 (1948).

carbon–nitrogen double bond.[11] The lifetime of the individual structures is estimated to be 0.4 sec at 25°.[25]

The dehydration of *N-tert*-butyl-2,2-dimethylsuccinamic acid with DCC yielded a compound with a camphor-like odor: mp 43–47°; ir 1810, 1712 cm^{-1}. This isoimide was very readily hydrolyzed and satisfactory analyses were not obtained. Similarly a crude preparation of *N*-(*p*-anisyl)succinisoimide was obtained, ir 1805, 1710 cm^{-1}, but attempts to isolate the pure compound were unsuccessful because of its ready hydrolysis.

A crude preparation of racemic *N-n*-butyl-2-methylsuccinisoimide was obtained from the corresponding amic acid by dehydration with ethyl chloroformate–triethylamine. Infrared analysis indicated the presence of isoimide (1805, 1700 cm^{-1}), but purification by preparative gpc produced the imide, ir 1755 weak, 1695 strong. *Anal.* Calcd for $C_{10}H_{15}O_2N$: C, 63.87; H, 8.93. Found: C, 63.65; H, 9.02.

The mono-*N-n*-butylamide of *cis*-cyclohexane-2,2-dicarboxylic acid was dehydrated with the ethyl chloroformate–triethylamine reagent; infrared analysis of the crude reaction mixture gave no evidence for the presence of isoimide. Purification of the crude mixture by preparative gpc produced the imide, ir 1720 cm^{-1} strong, 1790 cm^{-1} weak, *Anal.* Calcd for $C_{12}H_{19}O_2N$: C, 68.87; H, 9.15; N, 6.69. Found: C, 68.62; H, 9.40; N, 6.92.

Kinetic Studies. Triethylenediamine (Dabco) was recrystallized twice from acetone and was then sublimed and stored in a desiccator over P_2O_5. *N*-Methylimidazole was distilled and stored under nitrogen in the refrigerator. Glass-distilled water was used for the kinetic and product studies. Other chemicals were of reagent grade and were used without further purification.

Kinetic studies were carried out in a Gilford Model 240 recording uv spectrophotometer equipped with thermoplates. The temperature of the circulating bath was maintained at 25.0°. The reactions were run in 10% (v/v) acetonitrile–water with sufficient KCl added to make the total ionic strength equal to 0.21. The following buffers were used: HCl–KCl, HCl–sodium acetate, *N*-methylimidazole–HCl, Dabco–HCl, Tris–HCl, NaH_2PO_4–Na_2HPO_4, and $NaHCO_3$–Na_2CO_3. A Radiometer pH meter 26 equipped with a scale expander was used to measure pH.

The rate of the disappearance of isoimide **1a** in aqueous buffers was followed at 245 and 270 nm and the rate of the reactions of *N*-phenylphthalisoimide in aqueous HCl solutions was followed at 330 nm. Final absorbances were obtained after 10 or more half-lives and observed first-order rate constants were calculated from the integrated form of the first-order rate equation. The rate constants for individual runs agreed with the average of duplicate or triplicate determinations within ±6% or less.

Product Studies. Ultraviolet spectra of the 2,2-dimethylsuccinanilic acid and 2,2-dimethylsuccinanil were determined in various buffers. The position and intensity of the maxima (λ 242 nm (ε 11,700 ± 200)) did not vary significantly for the acid throughout the pH range studied. The absorbances of the completed reaction mixtures were determined for 5–10 wavelengths for several runs and the method of successive approximations was used to calculate the yields of amic acid and imide from the absorbance value at 242 nm. Further calculation of the expected absorbances for the appropriate imide–amic acid mixture at other wavelengths agreed with the measured absorbances within 2%.

Reaction of 1a with Phosphate and Tris Buffers. The reactions of isoimide **1a** with phosphate and Tris buffers were followed at 242 or 245 nm in the manner described above and were found not to be first-order reactions. Repeated scans of the wavelength regions from 230 to 330 showed a shift of λ_{max} from 253 to 242 nm and an increase in absorbance. This period was followed by a slower decrease in absorbance at 242 nm. For phosphate buffer at pH 7.96 with total buffer concentration 0.244 *M* the maximum absorbance at 242 was achieved at 15 min; the value for ε at that time was 9700, 83% of the value for pure amic acid at that wavelength. Similarly, with phosphate buffer at pH 8.16, total buffer concentration = 0.844 *M*, the maximum at 242 nm was reached after 6 min; ε was equal to 11,100 or 95% of the value for the amic acid at that wavelength. The final spectra for these solutions agreed with those calculated for amic acid–imide mixtures as reported in Table IV.

The phosphate-catalyzed reaction was monitored using a modification of the hydroxamic acid test.[26] Thus 1-ml samples of a reaction mixture, 0.01 *M* isoimide in 0.744 *M* phosphate buffer at pH 8.16 in 10% acetonitrile–water at 25°, were removed at various time intervals and were immediately added to 1 ml of hydroxylamine buffer (freshly prepared by mixing equal volumes of 4 *M* hydroxylamine hydrochloride and 3.5 *M* sodium hydroxide solutions). After 10 min 4 ml of 10% ferric chloride in 0.7 *N* HCl was added; the absorbance at 540 nm was read after an additional 10 min. The absorbance values dropped to 35% of that determined using the original isoimide solution as standard in 10 min; this period was followed by a gradual increase in absorbance values at 540 nm. For example, in one run the times and percentage absorbance values are as follows (time (min), $A_{540} \times 100/A_{540}$ (orig)): 1, 57; 3, 46; 5, 41; 7, 37; 10, 35; 21, 37; 33, 38; 49, 41; 60, 45. Both the isoimide **1a** and the imide **3a** gave positive hydroxamic acid tests; under the conditions described the intensity of the reading for the isoimide was *ca.* 80% of the intensity of the reading for the imide.

Rate constants for the Tris-catalyzed reaction extrapolated to zero buffer concentration at pH 7.95, 8.24, and 8.87 were 6.10×10^{-4}, 1.01×10^{-3}, and 8.10×10^{-3} sec^{-1}, respectively. There was considerable buffer catalysis of the second step which was more important at the higher pH's.

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(25) F. A. Bovey, "Nuclear Magnetic Resonance Spectroscopy, Academic Press, New York, N. Y., 1969, p 187.

(26) G. M. Blackburn and W. P. Jencks, *J. Amer. Chem. Soc.*, **90**, 2638 (1968).