Polyfunctional Catalysis. II. General Base Catalysis of the Mutarotation of Tetramethyl-D-glucose in Benzene and Methanol–Benzene

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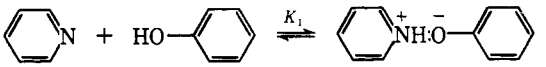
Abstract: Strong nucleophilic reagents such as diethylamine, phenoxide, 4-nitrophenoxide, and tetramethyl-D-glucoxide function in benzene or methanol–benzene as very effective general base catalysts for the mutarotation of 2,3,4,6-tetramethyl-D-glucose. The activation free energies for a series of nitro-substituted tetra-*n*-butylammonium phenoxides are directly proportional to the base strength of the catalysts. The addition of phenol to solutions of tetra-*n*-butylammonium phenoxide or 2,4-dinitrophenol to solutions of diethylammonium or triethylammonium 2,4-dinitrophenoxide has essentially no effect upon the catalytic activity of the ion pairs. Experiments with pyridine–phenol, pyridine–4-nitrophenol, diethylamine–phenol, diethylamine–4-nitrophenol, and diethylamine–2,4-dinitrophenol mixtures in benzene, methanol–benzene, or ether–benzene indicate that such systems function as general base catalysts through the action of the conjugate acid–base ion pairs. All of the experimental data reported to date on the mutarotation of tetramethyl-D-glucose in benzene are consistent with the interpretation that there is no concerted general acid–base reaction mechanism.

In the early fifties, Swain and Brown observed that the mutarotation of 2,3,4,6-tetramethyl-D-glucose (TMG) in benzene, as catalyzed by pyridine–phenol mixtures, gave a third-order rate expression

$$k_{ex} = (k + k')[\text{pyridine}][\text{phenol}] \quad (1)$$

where k_{ex} is the experimental pseudo-first-order rate constant.[1,2] In interpreting this result, they suggested that both the pyridine and the phenol acted upon the TMG in a concerted manner to give the product anomer (TMG′) directly, without the formation of either the conjugate acid or base of the sugar. This interpretation was accepted by most authors and reviewers and the reaction itself became the classic example of concerted general acid–base catalysis.[3] Pocker, however, proposed an alternative mechanism—a general base catalysis by a phenoxide ion within a pyridinium–phenoxide ion pair[4]



(2)

I

$$\text{I} + \text{TMG} \underset{k_0'}{\overset{k_0}{\rightleftharpoons}} \text{I} + \text{TMG}' \quad (3)$$

which also gives a third-order kinetic dependence

$$k_{ex} = (k_0 + k_0')[\text{pyridinium phenoxide}] = (k_0 + k_0')K_1[\text{pyridine}][\text{phenol}] \quad (4)$$

where K_1 is the equilibrium constant for the formation of the ion pair.

Since the mechanisms proposed by Pocker and by Swain and Brown are quite different from each other, it should be possible to perform experiments that would distinguish between them. For example, if Pocker's mechanism is correct, (a) compounds such as tetra-*n*-butylammonium phenoxide, tetra-*n*-butylammonium tetramethylglucoxide, and tetra-*n*-butylammonium 4-nitrophenoxide, which do not have a proton to donate, should be very effective catalysts for the mutarotation reaction; (b) other strong nucleophilic reagents (such as amines) should function in benzene in the absence of electrophilic reagents as very effective general base catalysts; and (c) mixtures of pyridine and strong phenolic acids (such as 2,4-dinitrophenol) should be relatively poor mutarotation catalysts. On the other hand, if Swain and Brown's interpretation is the correct one, just the reverse of the above should be observed: the compounds in items a and b should be poor catalysts and the mixture in item c should be an excellent catalyst.

To distinguish between these two mechanisms, we conducted a systematic series of experiments in which successive combinations of five bases—pyridine, diethylamine, tetra-*n*-butylammonium phenoxide, tetra-*n*-butylammonium 4-nitrophenoxide, and tetra-*n*-butylammonium 2,4-dinitrophenoxide—and three acids—phenol, 4-nitrophenol, and 2,4-dinitrophenol—were tested as catalysts in the mutarotation reaction. This paper reports the results of these experiments.

Experimental Section

Materials. Commercial 2,3,4,6-tetramethyl-D-glucose (Pierce Chemical Co.) was repeatedly vacuum sublimed in an Abderhalden-

(1) C. G. Swain and J. F. Brown, Jr., *J. Am. Chem. Soc.*, 74, 2534 (1952).

(2) C. G. Swain and J. F. Brown, Jr., *ibid.*, 74, 2538 (1952).

(3) (a) H. Lindley, *Advan. Enzymol.*, 15, 294 (1954); (b) D. E. Koshland, Jr., *ibid.*, 22, 82 (1960); (c) R. Lumry, *Enzymes*, 1, 216 (1959); (d) F. H. Westheimer, *ibid.*, 1, 301 (1959); (e) D. E. Koshland, Jr., *ibid.*, 1, 337 (1959); (f) F. M. Huennekens, "Investigation of Rates and Mechanisms of Reactions," Interscience Publishers, New York, N. Y., 1961, p 1234; (g) S. A. Bernhard, *ibid.*, p 617; (h) R. P. Bell, "The Proton in Chemistry," Cornell University Press, Ithaca, N. Y., 1959, p 153; (i) S. W. Benson, "The Foundations of Chemical Kinetics," McGraw-Hill Book Co., Inc., New York, N. Y., 1960, p 563; (j) M. L. Bender and R. Breslow, "Comprehensive Biochemistry," Vol. 2, Elsevier Publishing Co., Amsterdam, 1962, p 123; (k) J. Hine, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1956, p 243; (l) E. S. Gould, "Mechanisms and Structure in Organic Chemistry," Henry Holt and Co., New York, N. Y., 1959, p 111; (m) L. L. Ingraham, "Biochemical Mechanisms," John Wiley & Sons, Inc., New York, N. Y., 1962, p 25; (n) T. C. Bruice and S. Benkovic, "Bioorganic Mechanisms," Vol. I, W. A. Benjamin, Inc., New York, N. Y., 1966, p 40; (o) F. H. Dean, *J. Colloid Interfac. Sci.*, 24, 280 (1967); (p) H. R. Mahler and E. H. Cordes, "Biological Chemistry," Harper and Row, New York, N. Y., 1966, p 305; W. P. Jencks, "Catalysis in Chemistry and Enzymology," McGraw-Hill Book Co., Inc., New York, N. Y., 1969, p 199.

(4) Y. Pocker, *Chem. Ind.* (London), 968 (1960).

type sublimation apparatus (sketch available upon request) at the temperature of refluxing methanol and pressures below 2 Torr. Reagent 4-nitrophenol and 2,4-dinitrophenol (both Fisher) and phenol (Mallinckrodt) were vacuum sublimed, or, if the purification by sublimation had no effect upon the catalytic activity, used directly. Reagent 2-aminobutane and diethylamine (both Fisher) were employed before and after purification by repeated vacuum distillations. Practical 1,4-diazabicyclo[2.2.2]octane (DABCO) was recrystallized from benzene and sublimed. The remaining reagents —anhydrous reagent ether (Mallinckrodt), instrument grade benzene and methanol (Baker and Adamson), spectranalyzed benzene, reagent tetra-*n*-butylammonium hydroxide titrant solution in methanol, and several reagent grade amines (all Fisher)—were used directly without further purification. The concentration of the hydroxide titrant (nominally 0.79 *M*) was determined by titration (to a thymol blue end point) of a weighed amount of benzoic acid dissolved in a methanol.

Preparation of Solutions of Tetra-*n*-butylammonium Phenoxide, 4-Nitrophenoxide, and 2,4-Dinitrophenoxide. The ion pairs did not remain soluble in pure benzene, so mixed-solvent solutions were prepared from the stock titrant solution and 6% methanol–benzene solutions of phenol, 4-nitrophenol, and 2,4-dinitrophenol. Crystalline tetra-*n*-butylammonium 4-nitrophenoxide was prepared in the following manner: a 0.5 to 1% molar excess of 4-nitrophenol was dissolved in 5 ml of tetra-*n*-butylammonium hydroxide titrant and the resulting solution freeze dried; the solids were redissolved in 300 ml of hot benzene, and the yellow solution was decanted from the solid residue; this solution was slowly cooled and the resulting crystals filtered and dried. Analytical tests upon the resulting yellow crystalline solid were kinetic (*i.e.*, the mutarotation reaction) rather than thermal or spectroscopic.

Miscellaneous Details. The measurement and data-interpretation procedure and apparatus were identical with those previously reported.[5] Catalyst solutions were usually freshly prepared. The presence of methanol in the stock tetra-*n*-butylammonium hydroxide solution complicated the dilution procedures. Corrections to the experimental rate constants (usually not exceeding 5%) were made whenever it was difficult to prepare duplicate solution concentration levels. Mixed-solvent stock solutions were prepared by adding the appropriate amount of the minor solvent component (ether or methanol) to a volumetric flask and diluting to 100 ml with instrument grade benzene. Temperature control during the ion-pair experiments was improved to 25.00 ± 0.02°. Improvements in the optical purity of the tetramethylglucose (a result of the "Abderhalden" sublimation purification procedure) led to typical correlation coefficients for the pseudo-first-order experimental runs of 0.9998 or higher. The TMG concentration was 0.11 ± 0.01 *M*. At this concentration level, the "blank" mutarotation rate for different batches of TMG varied from 0.34×10^{-5} sec^{-1} to 1.2×10^{-5} sec^{-1} (most of the experiments were conducted with TMG that had a blank rate less than 0.64×10^{-5} sec^{-1}). In Tables II–IV, the term "rate constant" refers to the sum of the forward and reverse rate constants ($k + k'$).

General Base Catalysis

Amines. Swain and Brown reported that triethylamine was a relatively ineffective mutarotation catalyst ($k_{ex} = 23 \times 10^{-5}$ sec^{-1} for a 0.05 *M* solution) and that it participated in a general acid–base reaction mechanism in which TMG acted as the general acid.[2] We confirmed the fact that triethylamine was a poor catalyst, but also observed that primary and secondary amines were very active catalysts (Table I). For diethylamine, the experimental rate constant varied linearly with the amine concentration

$$k_{ex} = (k_1 + k_1')[\text{amine}] \qquad (4a)$$

and showed little evidence for significant catalyst–substrate complexing (Table II). From the data in Table II, the following activation parameters were calculated: $\Delta H^{\pm} = 10.4 \pm 1$ kcal/mol, $\Delta S^{\pm} = -28.3 \pm 3$ Gibbs/mol, and $\Delta G^{\pm} = 18.9 \pm 0.2$ kcal/mol. These values are given relative to the free amine and incorporate corrections of −1.38 Gibbs/mol and +0.41 kcal/mol in $\Delta S^{\pm}$ and $\Delta G^{\pm}$, respectively, to account for the fact that the rate constants for the forward and reverse anomerization steps are essentially the same.

Diethylamine and 2-aminobutane functioned as simple general base catalysts. There was no evidence for synergistic catalytic effects by electrophilic impurities in the amine, TMG, or solvent [the use of distilled or undistilled amine, different sources of benzene, a different solvent (CCl_4), and different batches of TMG had essentially no effect upon the mutarotation rate for

(5) P. R. Rony, *J. Am. Chem. Soc.*, 90, 2824 (1968).

Table I. Mutarotation of 2,3,4,6-Tetramethyl-D-glucose by Amines in Benzene[a]

Amine	Initial amine concn, *M*	Initial TMG concn, *M*	k_{ex}, 10^{-5} sec^{-1}
2-Aminobutane	0.0084	0.1098	208[b]
	0.0084	0.0995	215
	0.0084	0.424	169
	0.0126	0.1197	324
	0.0167	0.1221	441
Diethylamine	0.0100	0.1078	194[b]
	0.0060	0.1108	119[c]
	0.0060	0.1079	122[d]
	0.0060	0.1091	129[e]
	0.0060	0.1114	132[f]
1,4-Diazabicyclo[2.2.2]-octane	0.0025	0.1199	25
	0.0040	0.1186	38[g]
	0.0050	0.1195	49
	0.0100	0.1192	92
	0.0100	0.1199	93
	0.0100	0.1193	92[g]
	0.0200	0.1195	184
	0.0400	0.1188	273
	0.1000	0.1140	375[g]
Cyclohexylamine	0.0105	0.1067	275[b]
Benzylamine	0.0100	0.1079	239[b]
Ethanolamine	0.0100	0.1078	342[b]
Triethylamine	0.0101	0.1070	9.5[b]

[a] At 25.0°. Not corrected for blank. [b] Undistilled. [c] In spectroquality benzene. [d] In instrument grade benzene. [e] In spectroquality benzene containing 6.9 mg/ml of pot residue from West and Holden tetramethylglucose synthetic procedure [E. S. West and R. F. Holden, *Org. Syn.*, **20**, 97 (1940)]. [f] In instrument grade CCl_4. [g] Practical grade 1,4-diazabicyclo[2.2.2]octane. Unpurified.

Table II. Mutarotation of 2,3,4,6-Tetramethyl-D-glucose by Diethylamine in Benzene

Temp, °C	Initial catalyst concn, 10^{-5} *M*	Initial TMG concn, *M*	k_{ex}, 10^{-5} sec^{-1}
7.7	20	0.1045	1.15
8.0	1000	0.1055	63.1
8.0	2000	0.1080	120
8.1	3000	0.1062	167
25.0	40	0.1059	8.44
25.0	100	0.1118	20.7
25.0	300	0.1127	58.5
25.0	600	0.1108	119
25.0	603	0.1079	122
25.0	1000	0.1078	194
25.0	2000	0.1063	341
25.0	3000	0.1121	529
40.1	100	0.1144	47.1
40.1	300	0.1135	154
40.1	600	0.1145	260
40.0	1000	0.1073	408
40.0	2000	0.1103	811

Table III. Mutarotation of 2,3,4,6-Tetramethyl-D-glucose by General Bases[a]

Base	Solvent	Second-order rate constant,[b] M^{-1} sec^{-1}
Tetra-*n*-butylammonium 2,4-dinitrophenoxide	8.4% MeOH–Bz	0.0017[c]
Triethylamine[2]	Bz	0.005
Triethylamine[6]	Bz	0.006
Diethylammonium 2,4-dinitrophenoxide	6.0% MeOH–Bz	0.019
Triethylammonium 2,4-dinitrophenoxide[6]	Bz	0.021
Tetra-*n*-butylammonium 4-nitrophenoxide (solvated)[d]	8.4% MeOH–Bz	0.026[e]
	6.0% MeOH–Bz	0.028[f]
Diethylammonium 4-nitrophenoxide (solvated)[d]	4.0% ether–Bz	0.076[g]
1,4-Diazabicyclo[2.2.2]octane	Bz	0.099
Tetra-*n*-butylammonium 4-nitrophenoxide	8.4% MeOH–Bz	0.11[e]
	6.0% MeOH–Bz	0.15[f]
Diethylamine	Bz	0.19
	4.0% ether–Bz	0.19
	CCl_4	0.20
	6.0% MeOH–Bz	0.23
2-Aminobutane	Bz	0.26
Diethylammonium 4-nitrophenoxide	6.0% MeOH–Bz	0.91[h]
	4.0% ether–Bz	1.3[g]
Tetra-*n*-butylammonium phenoxide	5.7% MeOH–Bz	3.7
Tetra-*n*-butylammonium tetramethylglucoxide	5.7% MeOH–Bz	6.6
Diethylammonium phenoxide	6.0% MeOH–Bz	33[i]

[a] At 25.0°. Not corrected for blank. [b] Calculated either by a linear regression computer program or by the formula, k_{ex}/[base]. [c] Corrected for a solvent blank of 1.2×10^{-5} sec^{-1}. [d] Specifically solvated by one 4-nitrophenol molecule. Exact nature of solvation complex not determined. [e] Reactions 6–8 employed in computer analysis. Best fit corresponds to $K_2 = 59\ M^{-1}$ (correlation coefficient = 0.9987 for seven data points). [f] Same as *d*. Best fit corresponds to $K_2 = 83\ M^{-1}$ (correlation coefficient = 0.9997 for five data points). [g] Reactions 10–14 employed in computer analysis. Best fit corresponds to $K_2 = 77\ M^{-1}$ and $K_3 = 9\ M^{-1}$ (correlation coefficient = 0.99995 for seven data points). [h] Same as *g*. Best fit corresponds to $K_2 = 7\ M^{-1}$ and $K_3 = 12\ M^{-1}$ (correlation coefficient = 0.9987 for seven data points). Specie V has no catalytic activity (according to the computer analysis). [i] Value extrapolated from Figure 11.

either amine; excess TMG inhibited rather than accelerated the catalysis by 2-aminobutane (Table I)]. 1,4-Diazabicyclo[2.2.2]octane, a tertiary amine of low steric requirements, was a much more effective catalyst

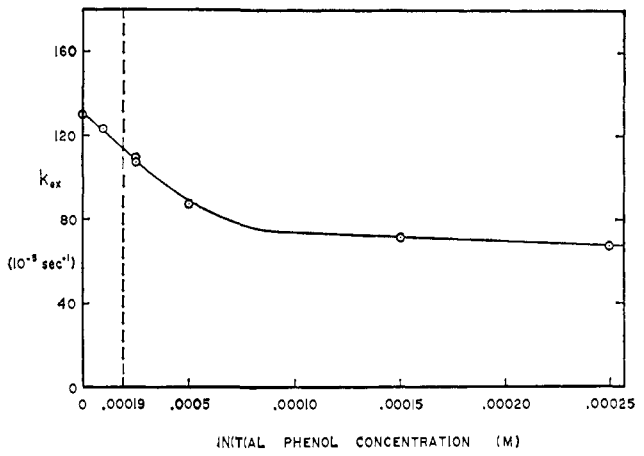
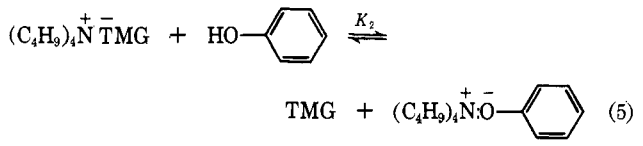


Figure 1. The experimental pseudo-first-order rate constant for tetra-*n*-butylammonium tetramethylglucoxide (0.000191 *M*) + phenol mixtures (in 5.7% methanol–benzene) as a function of the initial phenol concentration. The vertical dotted line represents the equivalence point for the titration of tetra-*n*-butylammonium tetramethylglucoxide by phenol.

than triethylamine, a sterically hindered tertiary amine. These experiments strongly supported the conclusion that there was a distinguishable general base mechanism for the mutarotation of tetramethylglucose in benzene.

Tetra-*n*-butylammonium Phenoxide and Tetramethylglucoxide. As a consequence of the leveling effect of tetramethylglucose, the strongest possible base in benzene solutions is the tetramethylglucoxide ion. Solutions of tetra-*n*-butylammonium tetramethylglucoxide were the most active mutarotation catalysts observed in dilute methanol–benzene solutions. Although the activity of this ion-pair catalyst was not studied as a function of concentration, it exhibited a second-order rate constant of 6.6 M^{-1} sec^{-1} at the single concentration level employed (Table III). The addition of phenol to methanol–benzene solutions of tetra-*n*-butylammonium tetramethylglucoxide caused a decrease in the observed reaction rate (Figure 1). The most likely explanation is an acid–base equilibrium



(5)

leading to the formation of a less active ion pair—tetra-*n*-butylammonium phenoxide—which has a second-order rate constant of 3.7 M^{-1} sec^{-1} at the single concentration level studied.

Tetra-*n*-butylammonium 4-Nitrophenoxide. The addition of 4-nitrophenol, a stronger acid than phenol, to solutions of tetra-*n*-butylammonium tetramethylglucoxide caused an even more substantial decrease in catalytic activity. The second-order rate constant for tetra-*n*-butylammonium 4-nitrophenoxide in 6% methanol–benzene was 0.15 M^{-1} sec^{-1} at the single concentration level studied (Table III).

Solutions of 0.0191 *M* tetra-*n*-butylammonium 4-nitrophenoxide containing excess 4-nitrophenol were prepared either by mixing dilute stock titrant solution with stock solutions of 4-nitrophenol, or else by adding

(6) A. Kergomard and M. Renard, *Tetrahedron Letters*, 769 (1968).

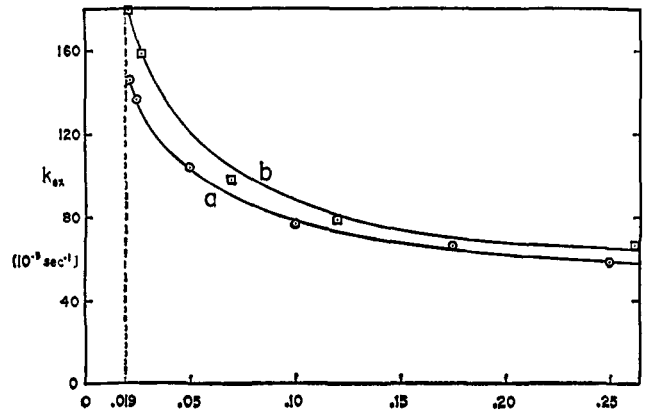
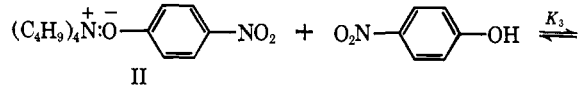


Figure 2. The experimental pseudo-first-order rate constant for tetra-*n*-butylammonium 4-nitrophenoxide (0.0191 *M*) + 4-nitrophenol mixtures [in 8.4% methanol-benzene (curve a) and 6% methanol-benzene (curve b)] as a function of the initial 4-nitrophenol concentration. Crystalline salt was used for the experiments in curve b. The vertical dotted line represents the equivalence point for the titration of tetra-*n*-butylammonium tetramethylglucoxide by 4-nitrophenol.

crystalline tetra-*n*-butylammonium 4-nitrophenoxide directly to the stock solutions of 4-nitrophenol. The results are shown in Figure 2 (where curve b corresponds to the crystalline salt). Different methanol concentration levels account for the observed difference in curves (methanol inhibits the mutarotation reaction). The vertical dotted line represents the equivalence point for the "titration" of tetra-*n*-butylammonium tetramethylglucoxide by 4-nitrophenol. The data points near this equivalence point were difficult to reproduce in curve b because any excess 4-nitrophenol that cocrystallized with the crystalline ion pair had a significant effect upon the experimentally observed rate constant.

Curves a and b were individually fitted by a linear regression analysis to the kinetic sequence



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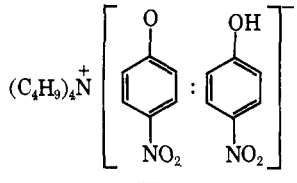

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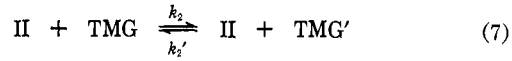

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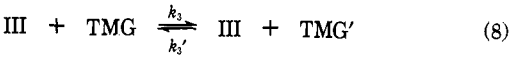


(8)

where III is a specifically solvated ion pair. The best fit for the two curves corresponded to values of K_3 of 59 M^{-1} (correlation coefficient = 0.9987 for seven data points) and 83 M^{-1} (correlation coefficient = 0.9997 for five data points), respectively. The specifically solvated ion pairs were less active than their nonsolvated counterparts (Table III).

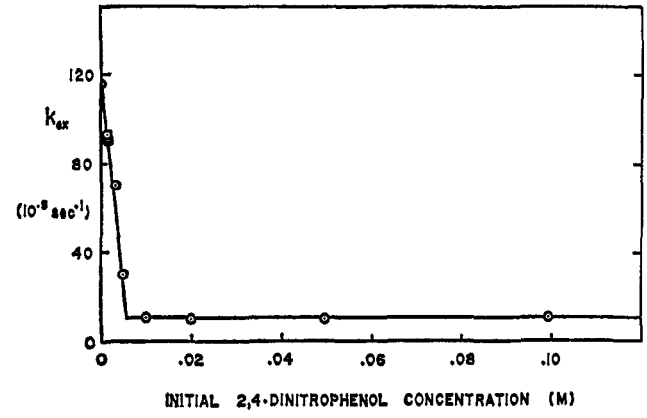


Figure 3. The experimental pseudo-first-order rate constant for diethylamine (0.005 *M*) + 2,4-dinitrophenol mixtures (in 6% methanol-benzene) as a function of the initial 2,4-dinitrophenol concentration.

It should be noted that the data in Figure 2 can also be fit to a third-order general acid–base reaction sequence (which is indistinguishable kinetically from reactions 6–8). We found no good reason to choose such a reaction mechanism.

Tetra-*n*-butylammonium 2,4-Dinitrophenoxide. The addition of 2,4-dinitrophenol to solutions of tetra-*n*-butylammonium tetramethylglucoxide almost eliminated all catalytic activity. The observed second-order rate constant for 8.4% methanol–benzene solutions containing 0.0191 *M* tetra-*n*-butylammonium 2,4-dinitrophenoxide was 0.0017 M^{-1} sec^{-1}, a factor of 4000 less than the rate constant initially observed for the tetra-*n*-butylammonium tetramethylglucoxide solution (Table III).

The addition of excess 2,4-dinitrophenol to methanol–benzene solutions of tetra-*n*-butylammonium 2,4-dinitrophenoxide produced a linear increase in the pseudo-first-order rate constant. This result, however, was due to *general acid* catalysis by 2,4-dinitrophenol (with a second-order rate constant in 6% methanol–benzene of 0.00035 M^{-1} sec^{-1}).

Acid–Base Catalytic Systems

Amines + 2,4-Dinitrophenol. Owing to the large formation constants in benzene and other nonpolar solvents,[7] amines stoichiometrically reacted with 2,4-dinitrophenol to form conjugate acid–base ion pairs. Both the amine and the conjugate base—the 2,4-dinitrophenoxide ion—were active mutarotation catalysts, so particularly striking catalytic effects were observed when 2,4-dinitrophenol was added to initially pure solutions of amine. For example, Figure 3 clearly shows the formation of an ion pair (diethylammonium 2,4-dinitrophenoxide) that is less active than the parent amine (diethylamine). Kergomard and Renard observed the opposite result: the formation of an ion pair (triethylammonium 2,4-dinitrophenoxide) that was more active than the parent amine (triethylamine) (Figure 4).[6] The diethylammonium 2,4-dinitrophenoxide ion pair was insoluble in pure benzene, so a mixed solvent consisting of 6% methanol–benzene was

(7) (a) R. G. Pearson and D. C. Vogelsong, *J. Am. Chem. Soc.*, **80**, 1038 (1958); (b) J. W. Bayles and A. Chetwyn, *J. Chem. Soc.*, 2328 (1958); (c) J. W. Bayles and A. F. Taylor, *ibid.*, 417 (1961); (d) E. F. Caldin and J. E. Crooks, *J. Chem. Soc., B*, 959 (1967).

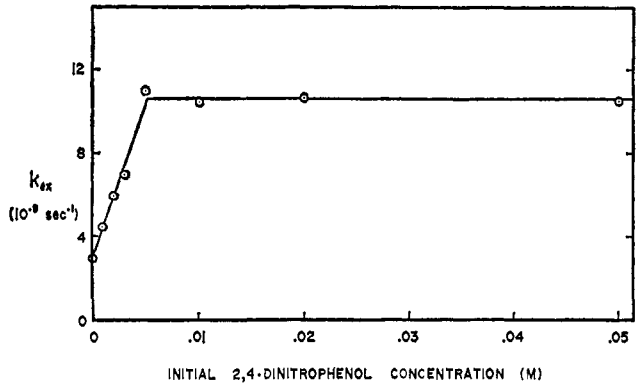


Figure 4. The experimental pseudo-first-order rate constant for triethylamine (0.005 M) + 2,4-dinitrophenol mixtures (in benzene) as a function of the initial 2,4-dinitrophenol concentration. The data is from Kergomard and Renard.[6]

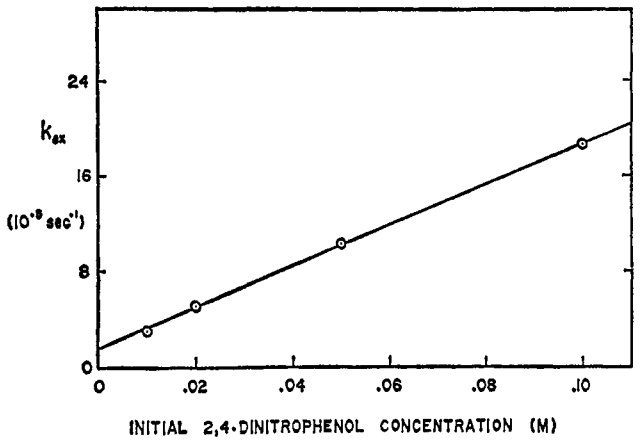


Figure 5. The experimental pseudo-first-order rate constant for pyridine (0.05 M) + 2,4-dinitrophenol mixtures (in 6% methanol–benzene) as a function of the initial 2,4-dinitrophenol concentration.

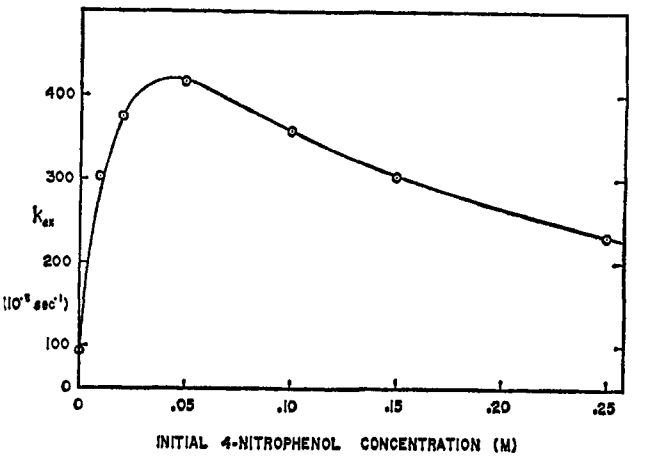


Figure 6. The experimental pseudo-first-order rate constant for diethylamine (0.005 M) + 4-nitrophenol mixtures (in 4% ether–benzene) as a function of the initial 4-nitrophenol concentration.

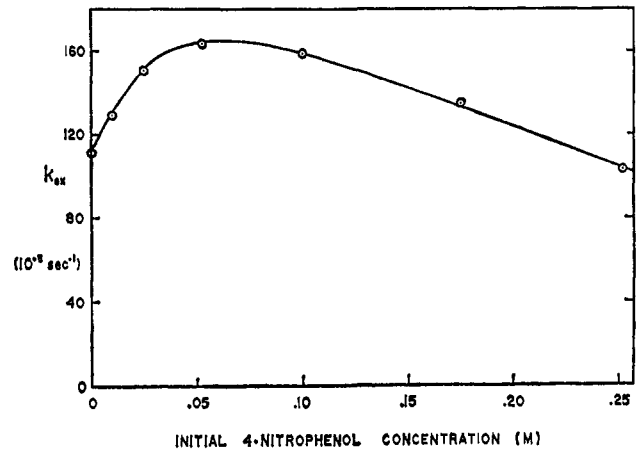


Figure 7. The experimental pseudo-first-order rate constant for diethylamine (0.005 M) + 4-nitrophenol mixtures (in 6% methanol–benzene) as a function of the initial 4-nitrophenol concentration.

required to keep solutions of the ion pair homogeneous (Kergomard and Renard didn't experience such difficulties, since mixtures of triethylamine and 2,4-dinitrophenol were soluble in benzene;[6] presumably the absence of active protons in the amine prevented the formation of insoluble ion-pair aggregates).

From the flat portions of the curves in Figures 3 and 4, the second-order rate constants for diethylammonium and triethylammonium 2,4-dinitrophenoxide were calculated to be 0.019 and 0.021 M^{-1} sec^{-1}, respectively. These values are greater than that observed for tetra-*n*-butylammonium 2,4-dinitrophenoxide (0.0017 M^{-1} sec^{-1}) and point to the fact that the nature of the cation in the ion pair affects the catalytic activity of the phenolic base.

Pyridine + 2,4-Dinitrophenol. The addition of excess 2,4-dinitrophenol to 0.05 M pyridine solutions in 6% methanol–benzene produced a linear increase in the observed rate constant (Figure 5). Fit to the following rate expression

$$k_{ex} = (k_4 + k_4')[\text{pyridine}][2,4\text{-dinitrophenol}] \quad (9)$$

the data gave a third-order rate constant of 0.034 M^{-2} sec^{-1} at the single pyridine concentration level studied (Table IV). The concentration of the conjugate acid–base ion pair, pyridinium 2,4-dinitrophenoxide, could not be obtained from an analysis of the kinetic data (if the ion pair were as active as diethylammonium 2,4-dinitrophenoxide, the observed rate constant in Figure 5 would climb to a limiting value of 95×10^{-5} sec^{-1}).

Table IV. Mutarotation of 2,3,4,6-Tetramethyl-D-glucose by Acid–Base Mixtures[a]

Base	Acid	Solvent	K_2, M^{-1}	Third-order rate constant, M^{-2} sec^{-1}
Pyridine	Phenol	Bz		0.018
Pyridine	4-Nitrophenol	6.0% MeOH–Bz	5.8	0.128[b]
Pyridine	2,4-Dinitrophenol	4.0% ether–Bz		0.034
Diethylamine	Phenol	Bz	12.5	11[c]

[a] At 25.0°. Not corrected for blank. [b] Correlation coefficient = 0.99999+ for four data points. [c] Correlation coefficient = 0.99955 for seven data points.

Diethylamine + 4-Nitrophenol. Experiments were conducted using 4% ether–benzene and 6% methanol–benzene solutions of 0.005 M diethylamine and excess 4-nitrophenol (Figures 6 and 7). Because insoluble

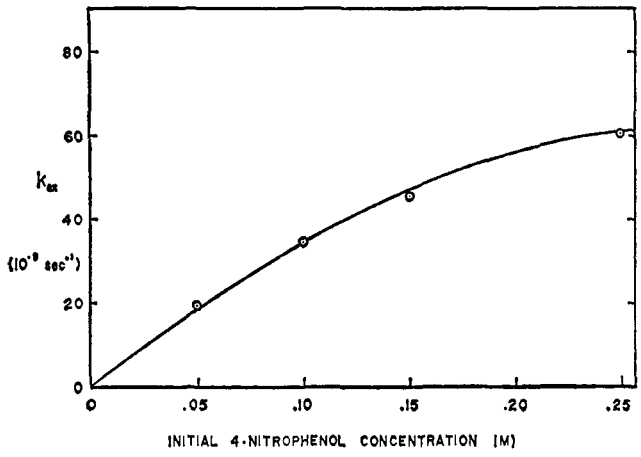


Figure 8. The experimental pseudo-first-order rate constant for pyridine (0.05 M) + 4-nitrophenol mixtures (in 4% ether–benzene) as a function of the initial 4-nitrophenol concentration.

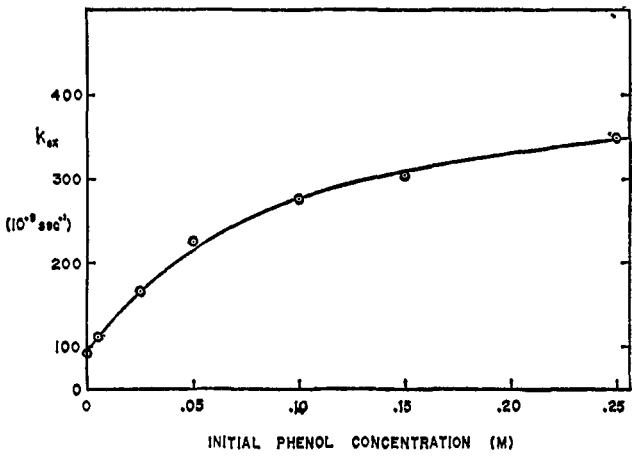
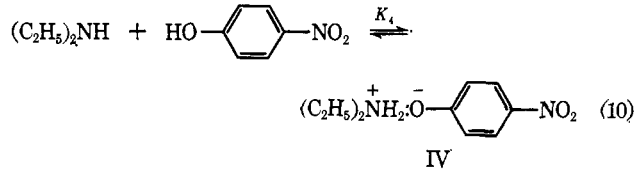


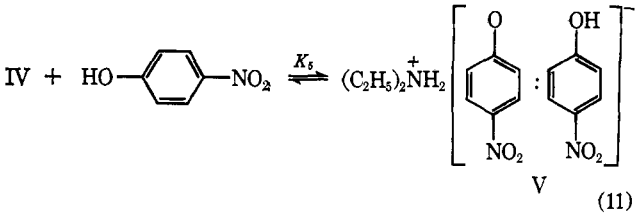
Figure 9. The experimental pseudo-first-order rate constant for diethylamine (0.005 M) + phenol mixtures (in benzene) as a function of the initial phenol concentration.

ion pairs also had a tendency to form in these systems, the presence of the methanol or ether kept the solutions homogeneous. The observed activity, however, was markedly dependent upon the nature of the cosolvent: the peak maximum was considerably higher for ether–benzene solutions (Figure 6) than for methanol–benzene solutions (Figure 7).

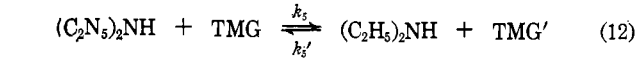
There were three active catalytic species in this acid–base system: diethylamine, the diethylammonium 4-nitrophenoxide ion pair, and the specifically solvated diethylammonium 4-nitrophenoxide ion pair. The seven data points in each figure were thus fitted by a linear regression analysis to a kinetic sequence consisting of reactions 10–14, where V is a specifically sol-



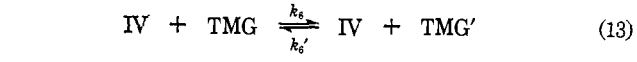
IV (10)



V (11)



(12)



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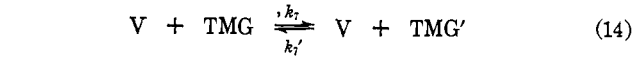
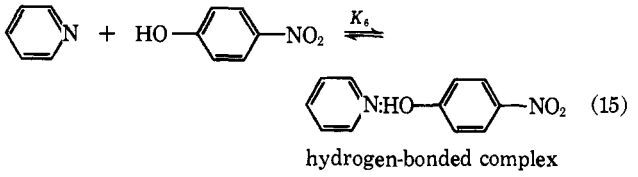


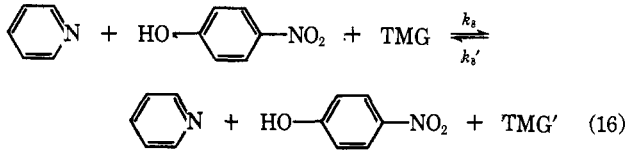
(14)

vated ion pair that is structurally similar to *III*. The correlation coefficient for Figure 6 was 0.99995. The values of the rate and equilibrium constants corresponding to this optimum fit are given in Table III. The data in Figure 7 did not fit the kinetic sequence nearly as well (correlation coefficient = 0.9987). Furthermore, the specifically solvated ion pair (according to the computer analysis) had no catalytic activity in 6% methanol–benzene, a conclusion that may be wrong.

Pyridine + 4-Nitrophenol. The addition of excess 4-nitrophenol to 0.05 M solutions of pyridine in benzene yielded the curve shown in Figure 8. The data was fit to the reaction sequence

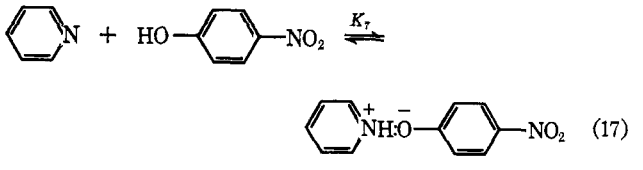


hydrogen-bonded complex (15)



(16)

and led to calculated equilibrium and third-order rate constants of 5.8 M^{-1} and 0.128 M^{-2} sec^{-1}, respectively (Table IV). The correlation coefficient for the four data points was a phenomenal 0.99999+, a result which demonstrates the value of the mutarotation reaction for the determination of equilibrium constants in nonpolar solvents. The concentration of the conjugate acid–base ion pair, pyridinium 4-nitrophenoxide, could not be calculated from the kinetic data. If the ion pair were as active as diethylammonium 4-nitrophenoxide,



(17)

its formation constant, K_7, would be approximately 0.1 M^{-1}.

Diethylamine + Phenol. The addition of excess phenol to 0.005 M solutions of diethylamine in benzene yielded a curve similar to Figure 8 (Figure 9). Fit to the reaction sequence

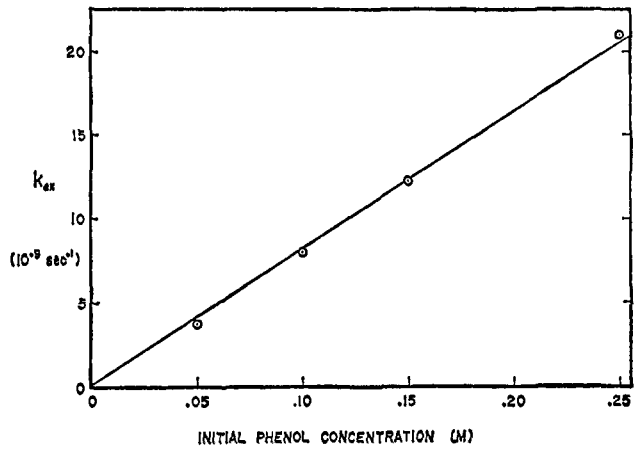


Figure 10. The experimental pseudo-first-order rate constant for pyridine (0.05 M) + phenol mixtures (in benzene) as a function of the initial phenol concentration.

$$(C_2H_5)_2NH + HO\text{—}C_6H_4 \overset{K_a}{\rightleftharpoons} (C_2H_5)_2NH{\cdot}HO\text{—}C_6H_4 \quad (18)$$

hydrogen-bonded complex

$$(C_2H_5)_2NH + TMG \overset{0.187\ M^{-1}\ sec^{-1}}{\rightleftharpoons} (C_2H_5)_2NH + TMG' \quad (19)$$

$$(C_2H_5)_2NH + HO\text{—}C_6H_4 + TMG \overset{k_3}{\underset{k_3}{\rightleftharpoons}}$$

$$(C_2H_5)_2NH + HO\text{—}C_6H_4 + TMG' \quad (20)$$

the data gave an equilibrium constant of 12.5 M^{-1}, a third-order rate constant of 11 M^{-2} sec^{-1}, and a correlation coefficient of 0.99955 for the seven data points. The concentration of diethylammonium phenoxide could not be calculated from the kinetic data.

Pyridine + Phenol. The addition of excess phenol to 0.05 M solutions of pyridine in benzene gave the linear curve shown in Figure 10. The data corresponds to the rate expression

$$k_{ex} = (k_{10} + k_{10}')[\text{pyridine}][\text{phenol}] \quad (21)$$

and a third-order rate constant of 0.018 M^{-1} sec^{-1}. We observed no evidence for the formation of significant quantities of the pyridine–phenol hydrogen-bonded complex.[8] The pyridinium phenoxide ion pair, whose concentration was not directly measured, is probably more active than tetra-*n*-butylammonium tetramethylglucoxide.

Benzene solutions of 4-benzylpyridine (0.10 M) and 4-*t*-butylphenol (0.12 M) were studied in benzene in an effort to determine whether a sandwich-type pyridine–phenol configuration existed in the transition state (the bulky substituents would prevent the formation of such a configuration). The rate constant obtained (17.1 $\times$ 10^{-5} sec^{-1}) was nearly identical with the value previously obtained for the pyridine–phenol system.[5] The data thus suggest that a sandwich-type configuration does not exist.

Pyridine + Thiophenol. A mixture of pyridine (0.05 M) and undistilled thiophenol (0.10 M) was also screened for catalytic activity in the mutarotation reaction. The observed rate constant, 0.47 $\times$ 10^{-5} sec^{-1}, was essentially equal to the blank mutarotation rate, 0.60 $\times$ 10^{-5} sec^{-1}. Since the thiophenoxide ion is a much weaker base than the phenoxide ion, this result is consistent with Pocker's mechanism.

(8) Figure 3 in ref 5 has an error: $\Delta G = +1.9$ should be replaced by $\Delta G = -1.9$. Reactions 9 and 9′ are probably incorrect, but a better kinetic sequence has not yet been found.

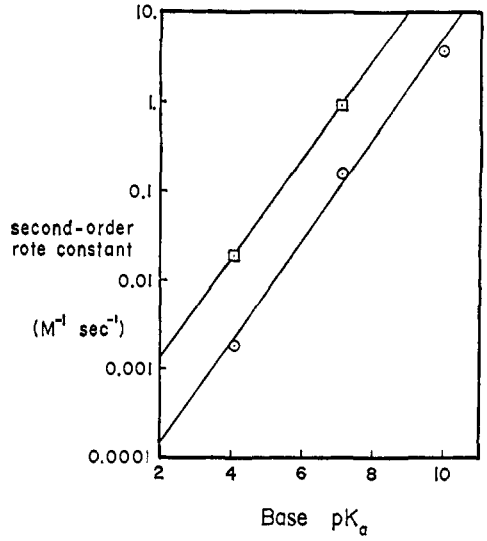


Figure 11. The calculated second-order rate constants for a series of nitro-substituted diethylammonium (⊡) and tetra-*n*-butylammonium (⊙) phenoxides as a function of base pK_a in water.

Discussion

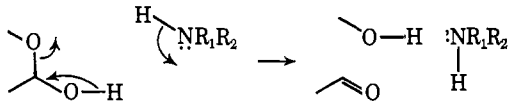
From the results reported in this paper, we conclude that strong nucleophilic reagents, such as amines and substituted alkylammonium phenoxides, function in benzene and methanol–benzene *in the absence of electrophilic reagents* as very effective general base catalysts for the mutarotation of 2,3,4,6-tetramethyl-D-glucose. For a series of nitro-substituted tetra-*n*-butylammonium phenoxides, the activation free energies at 25° were directly proportional to base strength (Figure 11). The experimentally determined second-order rate constants spanned four powers of ten at 25°, from very weak bases such as tetra-*n*-butylammonium 2,4-dinitrophenoxide to very strong bases such as tetra-*n*-butylammonium tetramethylglucoxide. The addition of a molar excess of strong acids to these ion-pair catalysts either had no effect or else caused a *decrease* in the observed catalytic activity. An excess of methanol or tetramethylglucose also inhibited the mutarotation reaction.

Steric effects appear to play an important role in the general base catalysis of the mutarotation of tetramethylglucose. For example, 1,4-diazabicyclo[2.2.2]octane, though a weaker base (pK_a = 9.6)[9,10] (but a sterically less hindered tertiary amine) than triethylamine (pK_a = 10.7),[11] is a much more effective catalyst. Nitro-substituted alkylammonium and pyridinium phenoxides exhibited an order of activity

$$\overset{+}{N}H > (C_2H_5)_2\overset{+}{N}H_2 > (C_4H_9)_4\overset{+}{N}$$

(9) J. Hine, J. G. Houston, J. H. Jensen, and J. Mulders, *J. Am. Chem. Soc.*, 87, 5050 (1965).
(10) H. Anderson, C. Su, and J. W. Watson, *ibid.*, 91, 482 (1969).
(11) H. A. Sober, "Handbook of Biochemistry," The Chemical Rubber Co., Cleveland, Ohio, 1968, p J-168.

that is consistent with a steric interpretation. Primary and secondary amines do not appear to act as simultaneous proton donor–acceptors.



Experimental evidence that points to such an interpretation can be equally well explained on the basis of steric effects.

The experimental data thus strongly suggest that pyridine–phenol, pyridine–4-nitrophenol, diethylamine–phenol, and other acid–base mixtures function as general base catalysts through the action of the conjugate acid–base ion pairs. Owing to traditional difficulties in distinguishing between different mechanisms that contain identical transition states except for the distribution of atoms, it is very difficult to unequivocally disprove the existence of a concerted general acid–base mechanism in the mutarotation reaction. All of the experimental data reported to date are consistent with the interpretation that there is no such mechanism.[1,2,4–6]

Acknowledgments. The authors gratefully acknowledge numerous stimulating discussions with Professor Jack Halpern and Professor Richard L. Schowen.

Studies of the Chymotrypsinogen Family of Proteins. VI. Characterization of the Conformational Variation of Chymotrypsin[1]

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Received July 31, 1968

Abstract: The structural variation of α-chymotrypsin has been studied in the acid pH region and is shown to consist of two distinct equilibrium situations. The first of these, transition $A_a \rightarrow A_b$, is a thermodynamically small structural change and is described by changes in the optical rotatory dispersion. The second, transition I, is a thermodynamically large conformational change which is uniquely described by changes in the ultraviolet absorption spectrum. It is shown that transition I at pH 2.0 is a strongly cooperative, two-state transition and that the thermodynamic behavior is consistent with the Brandts model of protein unfolding.

Considerable recent effort has been focused on the thermodynamic changes occurring during polypeptide and protein conformational transitions as a means for obtaining information about the globular protein in solution and an understanding of the thermodynamic determination of the conformation. Of particular significance is the work of Brandts who has been successful in describing the thermally induced unfolding of chymotrypsinogen A[2,3] and ribonuclease[4] in terms of a relatively simple model assuming that only two unique thermodynamic states of the protein exist under experimental conditions. We now wish to apply this analysis to chymotrypsin and a variety of its chemical derivatives for the purposes of testing the method of Brandts and extending it as a phenomenological tool to aid in the understanding of protein thermodynamic stability. In addition, this type of analysis of the chymotrypsinogen family is necessary for any fundamental understanding of chymotryptic catalysis.

The application of the Brandts method of analysis requires that the conformational transition under consideration be well approximated by simple equilibrium between two distinct thermodynamic states. This is necessary in order to obtain meaningful thermodynamic quantities from the experimental data. Although Brandts was able to demonstrate two-state behavior in the unfolding transition of chymotrypsinogen in a straightforward manner using ultraviolet absorbance, the situation for chymotrypsin is more complicated, as is indicated by an apparent discrepancy between the optical rotatory dispersion (ORD) and ultraviolet absorption changes for the thermal transition at pH 2.0.[5] Therefore, before considering in detail the thermodynamics of the thermal transition of chymotrypsin, it is necessary to describe the conformational states of this protein in the acid to neutral pH region.

A clue to the source of difficulty in chymotrypsin was provided by the observation by Rupley, Dreyer, and Neurath[6] that this protein exhibits a marked variation in optical activity with pH. Subsequently, it was shown that these changes were due primarily to changes in a single Cotton effect centered near 207 nm.[7] Parker attributed this change to the rupture of an ion pair stable only in the pH region from 3 to 9.[8] Such an ion pair in

(1) This is paper no. 39 from this laboratory. Please request reprint by this number. The work in this paper is from the Ph.D. Dissertation of R. Biltonen, University of Minnesota, 1965.
(2) J. Brandts, *J. Am. Chem. Soc.*, **86**, 4291 (1964).
(3) J. Brandts, *ibid.*, **86**, 4302 (1964).
(4) J. Brandts, *ibid.*, **87**, 2759 (1965); J. Brandts and L. Hunt, *ibid.*, **89**, 4826 (1967).
(5) B. Havsteen, B. Labouesse, and G. P. Hess, *ibid.*, **85**, 796 (1963).
(6) J. Rupley, W. Dreyer, and H. Neurath, *Biochem. Biophys. Acta*, **18**, 162 (1955).
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(8) H. Parker, Dissertation, University of Minnesota, 1967.