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A Comparison of the Bimolecular and Intramolecular Nucleophilic Catalysis of the Hydrolysis of Substituted Phenyl Acylates by the Dimethylamino Group

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The rate constants and activation parameters have been determined for the nucleophilic displacement of substituted phenols from phenyl acylates (solv. H_2O) in the systems I [trimethylamine + *m*- and *p*-substituted phenyl acetates], II [*m*- and *p*-substituted phenyl γ-(N,N-dimethylamino)-butyrates] and III [*m*- and *p*-substituted phenyl δ-(N,N-dimethylamino)-valerates]. The values of $\Delta H^{\ddagger}$ were found to be, within experimental error, invariant for all the esters of system I, II and III. Therefore, all electronic and steric factors—both being large—are reflected in $\Delta S^{\ddagger}$, providing linear free energy relationships (unusually) dependent only on $\Delta S^{\ddagger}$. The $OH^{\ominus}$-catalyzed hydrolysis of *m*- and *p*-substituted O-acetylphenols was also found to be characterized by a constant $\Delta H^{\ddagger}$ value and a substituent controlled $\Delta S^{\ddagger}$. The unimolecular processes of II and III involving five- and six-membered transition states had $T\Delta S^{\ddagger}$ higher by 3.6 ± 0.6 and 4.7 ± 1.1 kcal. mole^{-1}, respectively, than the bimolecular process of I. The Hammett ρ-values for I, II and III are very nearly identical (+2.2, +2.5, +2.5, respectively). The constancy of $\Delta H^{\ddagger}$ and ρ- for systems I, II and III as well as the uniform dependence of $\Delta\Delta S^{\ddagger}$ on the nature of the substituent group provides convincing evidence that the bimolecular and unimolecular processes take place by the same mechanism. The ratio of rate constants for the similarly substituted esters of II *vs.* those of III was found to be 2.3. This value is but $^1/_{100}$ that previously determined as the ratio for the formation of glutaric and succinic anhydride in the intramolecular carboxyl anion nucleophilic catalysis of the hydrolysis of monophenyl glutarates and succinates. This difference as well as the apparent change in mechanism previously noted for the conversion of carboxyl anion catalysis of substituted phenyl acetates to an intramolecular process are discussed.

Introduction

Systems involving intramolecular participation of acidic and basic groups in ester and amide hydrolysis have drawn attention as partial models for hydrolytic enzymes.[2] The intramolecular "models" and the enzymatic reactions share in common the bringing together of reactant species within a single molecule or complex, respectively (propinquity effect). The enhancement in efficiency obtained by the propinquity factor and considerations as to whether this might account in great part for the facility of enzymatic processes have been discussed.[3-6] The only means of ascertaining the kinetic significance of the propinquity effect is through the study of reactions occurring intramolecularly or *via* pre-equilibrium complex formation. In the conversion of a bimolecular reaction into a unimolecular process the possibility arises that the details of mechanism or the actual mechanism of the reaction might be altered. Thus, the bringing together of groups in an intramolecular or an enzymatic reaction may alter the course of the reaction by favoring reaction mechanisms which may not be observable in simple bimolecular reactions (change of mechanism); or the bringing together of groups may change the nature of a reaction by causing a step to become rate determining which in the bimolecular process would not be so (change of rate-limiting step). In order to ascertain the importance of these possibilities we have initiated studies of intramolecular catalysis of the hydrolysis of esters and amides.[7-14] To determine if and how a mechanism is altered on bringing reactant groups into close proximity it is desirable to have available as many measurable parameters for the reactions as possible. The determination of $\Delta F^{\ddagger}$, $\Delta H^{\ddagger}$, $T\Delta S^{\ddagger}$ and ρ for a series of intermolecular reactions and their intramolecular counterparts has been difficult due to competing side reactions in one or another of the processes, instability of compounds, etc. The present study is concerned with a comparison of the three systems of Chart I. The tertiary amine was chosen as the nucleophile on the basis of its pK_a'—assuring readily measurable rates in both inter- and intramolecular processes—and its inability to partake in complicating general base-catalyzed attack at the ester carbonyl group, as can secondary and primary amines.[15-17]

Experimental

Compounds. **Trimethylamine hydrochloride** (Eastman Kodak Co., white label) was recrystallized from methanol and dried over P_2O_5. ***p*-Nitro-, *m*-nitro-, *p*-chloro- and *p*-methyl-O-acetyl phenols** as well as **O-acetylphenol** itself were from a former study[18] and were repurified by crystallization or distillation prior to use.

γ-(N,N-Dimethylamino)-butyric Acid Hydrochloride.—To 25.0 g. (0.24 mole) of γ-aminobutyric acid was added 63 ml. of 90% formic acid (1.50 moles) and 48.5 ml. of 37% aqueous formalin solution (0.49 mole). The resultant solution was refluxed

(1) NIH Predoctoral Fellow (1961—present). Part of the work to be submitted by S. J. Benkovic in partial fulfillment for the Ph.D. degree, Cornell University.

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(17) T. C. Bruice and M. F. Mayahi, *ibid.*, **82**, 3067 (1960).

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for 16 hr., cooled, and acidified with 28 ml. of concentrated hydrochloric acid. Evaporation to dryness yielded a white crystalline solid which was recrystallized from acetonitrile to give the pure amino acid hydrochloride in 70% yield, m.p. 148–149° (lit.[19] 145–147°).

δ-(N,N-Dimethylamino)-valeric Acid Hydrochloride.—To 10.0 g. (0.085 mole) of δ-aminovaleric acid was added 22 ml. of 90% formic acid (0.525 mole) and 25.4 ml. (0.255 mole) of 37% formalin solution. The amino acid hydrochloride was obtained in 85% yield as in the previous case after recrystallization from acetonitrile; m.p. 166–167°. (lit.[20] 163–165°).

The substituted phenyl esters of γ-(N,N-dimethylamino)-butyric and δ-(N,N-dimethylamino)-valeric acids were prepared by the general method detailed below. To a small test-tube containing 1.0 g. (0.006 mole) of the γ-(N,N-dimethylamino)-butyric acid hydrochloride or 1.0 g. (0.0055 mole) of the δ-(N,N-dimethylamino)-valeric acid hydrochloride was added 3.0 ml. (0.021 mole) of trifluoroacetic anhydride. The tube was tightly stoppered with a calcium chloride drying tube and warmed at 35° for 20 minutes. The cooled homogeneous clear solution was then saturated with dry hydrogen chloride. To this was added the desired phenol (in 1.8 mole excess to the acid hydrochloride), and with the drying tube in place the resulting mixture was heated in an oil-bath with occasional agitation at 55° for 1.5 hr. The resulting clear solution was cooled and layered with ether (dried over sodium) causing either crystallization or oiling out of the ester. By grinding the oil under anhydrous ether there was obtained the crude ester hydrochloride in 50–80% of theory. The crude products were recrystallized from 3:1 (*v./v.*) chloroform–carbon tetrachloride solution by layering said solution with anhydrous ether. The recrystallized products were dried over P_2O_5. The ester hydrochlorides so obtained were found to be extremely hygroscopic and sensitive to heat and light, necessitating immediate analysis. Chloride determinations were carried out by the Volhard procedure, nitrogen analysis by the Kjeldahl procedure, and phenoxy group analysis were carried out spectrophotometrically by comparing the t_∞ absorbance of a solution of the ester in *p*H 9.5 borate buffer to the absorbance of standard curves of the phenolate ions in *p*H 9.5 borate buffer. The analytical data are provided in Table I.

TABLE I

MELTING POINTS AND ANALYTICAL DATA FOR THE PHENYL ESTERS OF γ-(N,N-DIMETHYLAMINO)-BUTYRATE HYDROCHLORIDE AND δ-N,(N-DIMETHYLAMINO)-VALERATE HYDROCHLORIDE

Cmpd. (Butyrate series)	M.p., °C.	Calcd., % x-C_6H_4-OH, Cl^-, N	Found, % x-C_6H_4-OH, Cl^-, N
$C_6H_5NO_2$-*p*	89–90	48.2, 12.28, 9.70	47.3, 12.11, 9.31
C_6H_5Cl-*p*	134–135	46.2, 12.75, 5.04	46.1, 12.51, 4.95
C_6H_5H	87–88	38.6, 14.75, 5.74	38.1, 14.55, 5.56
$C_6H_5CH_3$-*p*	126–127	42.0, 13.75, 5.43	41.5, 13.77, 5.27
(Valerate series)			
$C_6H_5NO_2$-*p*	97–98	46.0, 11.71, 9.25	46.5, 11.52, 8.97
C_6H_5Cl-*p*	151–152	44.0, 12.14, 4.79	43.8, 11.91, 4.92
C_6H_5H	105–106	36.5, 13.75, 5.43	36.7, 13.71, 5.28
$C_6H_5CH_3$-*p*	150–151	39.8, 13.05, 5.15	39.2, 12.75, 4.91
(Butyrate series)		C, H, N	C, H, N
m-NO_2[a]	127–128	49.65, 5.93, 9.70	49.07, 6.03, 9.88
(Valerate series)			
m-NO_2[a]	115–116	51.55, 6.32, 9.25	51.04, 6.60, 9.05

[a] Midwest Micro Labs., Indianapolis, Ind.

Kinetic Measurements. *Intramolecular* displacement of phenoxide ions from the phenyl esters of γ-(N,N-dimethylamino) butyric and δ-(N,N-dimethylamino)-valeric acids was followed by means of stopped-flow spectrophotometry. Buffer solutions were of a calculated ionic strength of 1.0 *M*—though the reactions are insensitive to ionic strength. Buffers were prepared by combining solutions A with solutions B in the proper ratio:

*p*H range	A	B
5–7	0.33 *M* KH_2PO_4	0.33 *M* K_2HPO_4
	.67 *M* KCl	.01 *M* KCl
7–9.5	.40 *M* KH_2BO_3	.40 *M* H_3BO_3
	.60 *M* KCl	1.0 *M* KCl
9.5–11	.20 *M* K_2HPO_4	0.20 *M* K_3PO_4
	.40 *M* KCl	0.40 *M* KCl

For each experiment, equal volumes of a 1.0 *M* KCl solution $\sim 5 \times 10^{-4}$ *M* in appropriate ester and the correct buffer were thermostated in the reservoir syringes, transferred to the thermostated stopped-flow syringes (±0.2°) and mixed in the stopped-flow cell. In the case of the *m*-and *p*-nitro esters the 1.0 *M* KCl solution was acidified to *ca.* *p*H 2.0 prior to solution of the ester to minimize its solvolysis. The wave lengths employed to follow the formation of phenoxide ions were

Substituent	*p*-CH_3	H	*p*-Cl	*m*-NO_2	*p*-NO_2
mμ	280	275	285	350	330

The *p*H of the run was taken as that of the spent solution ejected from the stopped-flow cell compartment at t_∞. Five to twenty runs were made at each *p*H and at each temperature. Reported rate constants have been calculated from at least five superimposable recordings of phenol release *vs.* time. The reactions were all found to follow first-order kinetics with good accuracy and the method employed to calculate the values of the rate constants was that of Guggenheim.[21]

Bimolecular Reactions.—Solutions of trimethylamine-trimethylamine hydrochloride were prepared immediately before use by half-neutralization of solutions of the hydrochloride with standardized potassium hydroxide solution. All solutions were maintained at a calculated ionic strength of 1.6 *M* with KCl. The stock solutions so prepared were kept at 0 ° in an ice-bath. Dilution of the stock solutions to obtain the correct amine concentration were made with 1.6 *M* μ buffer (0°) of the same *p*H as the amine solution. Amine concentrations of 0.15 to 1.5 *M* were employed. The various amine solutions were then drawn into 30-ml. Luer-lock syringes equipped with stopcocks, all air displaced and the syringes replaced in the ice-bath until initiation of the kinetic run. Prior to each run the amine solutions were injected into a stoppered cuvette and allowed to equilibrate in a constant temperature cell block fitted to the spectrophotometer. The reaction was initiated by adding 2 drops of the solution of ester from a calibrated dropper to the cuvette resulting in a final ester concentration of approximately 3×10^{-4} *M*. Formation of phenolate ion was followed by recording the rate of change of absorption at the wave lengths

Substituent	H	*p*-Cl	*m*-NO_2	*p*-NO_2
mμ	275	285	420	400

The pseudo-first-order rate constants (k_{obs}) were determined as the slopes of plots of log (O.D.$_\infty$ − O.D.$_t$) *vs.* *t*.

pK_a' Determinations.—The pK_a' of the dimethylamino group for the *p*-CH_3, H and *p*-Cl substituted phenyl esters of γ-(N,N-dimethylamino)-butyric and δ-(N,N-dimethyl)-valeric acids at 20° were assumed to be those of the pK_{app} value determined kinetically from the *p*H–rate profiles. In a similar manner the pK_a' values for the phenyl γ-(N,N-dimethylamino)-butyrate and *p*-cresol δ-(N,N-dimethylamino)-valerate esters at 10° and 34° were assumed to be those of the pK_{app}' values determined at these temperatures from *p*H–rate profiles.

The pK_a' of trimethylamine at each temperature was obtained by the method of half neutralization; $pK_a'_{34°} = 10.00$, $pK_a'_{20°} = 10.28$, $pK_a'_{10°} = 10.50$.

Apparatus.—All *p*H measurements were made with a Radiometer model 22 *p*H meter using a combined Radiometer GK2021 electrode thermostated (±0.1°) at the same temperature as the accompanying kinetic reactions. The rates of liberation of substituted phenolate ions from the esters were followed with a Zeiss PMQII spectrophotometer. For the slower reactions the spectrophotometer was fitted with a special hollow brass cuvette holder thermostated at 30 ± 0.01° by a Haake constant temperature circulating bath. For the more facile reactions the spectrophotometer was equipped with a stopped-flow block designed around the model employed by Sturtevant.[22] In our modified design the plunger syringes as well as the reservoir syringes were imbedded in a thermostated brass block. Also imbedded in the block were the mixing and observation cell (Plexiglass with quartz windows) and the channels (Tygon). The temperature within the brass-block as well as that within the observation cell were measured *via* thermistor probes. The change of absorbance with time was recorded on a Brush model RD2321-00 inking oscillograph recorder.

Results

Intramolecular Reactions.—In the solvolysis of systems II and III, the rate of phenolate ion formation would be expected to be due to intramolecular nucleophilic displacement at the ester bond by the unprotonated dimethylamino group (k_1) and hydrolysis by

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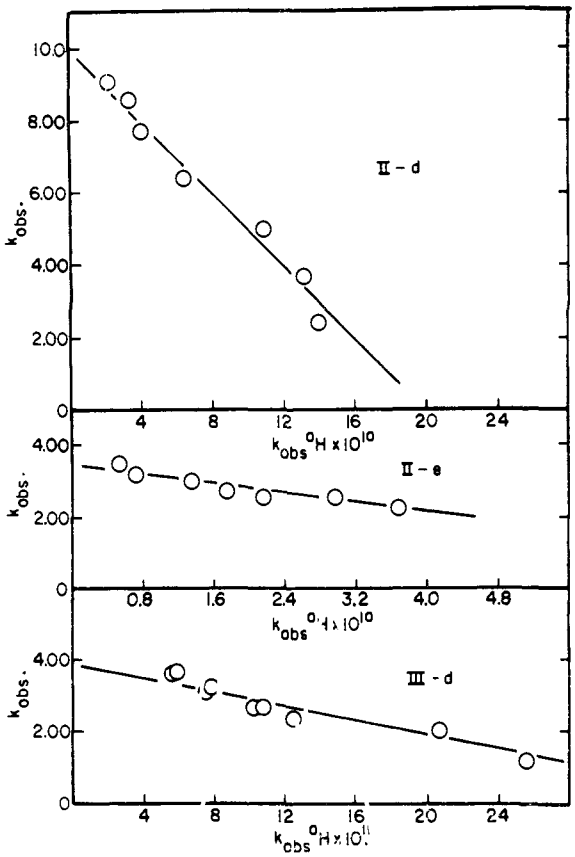


Fig. 1.—Plots of k_{obs} *vs.* $k_{obs}\,a_H$ for the intramolecular nucleophilic catalysis of the hydrolysis of phenyl γ-(N,N-dimethylamino)-butyrate (II-d), *p*-methylphenyl γ-(N,N-dimethylamino)-butyrate (II-e) and phenyl δ-(N,N-dimethylamino)-valerate (III-d) in water (μ = 1.0) at 20°.

lyate species (k_0)

$$\text{d phenol}/dt = k_0 C_{E_T} + k_1 C_E \qquad (1)$$

where C_{ET} is the total concentration of ester (*i.e.*, $C_{ET} = C_E + C_{EH^\oplus}$). At any *p*H the values of the observed first-order appearance of phenolate ion (k_{obs}) would then be given by 2 and 3

$$k_{obs} = k_0 + k_1\left(\frac{K_a'}{K_a' + a_H}\right) \qquad (2)$$

$$k_{obs} - k_0 = k_1 - \frac{1}{K_a'}(k_{obs} - k_0)a_H \qquad (3)$$

where K_a' is the dissociation constant for the dimethylammonium group and a_H the hydrogen ion activity, as determined by the glass-electrode. Plots of k_{obs} *vs.* $k_{obs}\,a_H$ were found to be linear for both the phenyl and *p*-cresol esters of II and III (Fig. 1). Thus, k_0 is insignificant for these esters. Since the ρ value for nucleophilic displacement on phenyl esters by amines is about twice that for nucleophilic displacement by $OH^\ominus$ and 100 times greater than displacement by $H_3O^\oplus$[17] it is safe to assume k_0 may also be ignored for the *p*-Cl, *p*-NO_2 and *m*-NO_2 esters of II and III. From the plot of k_{obs} *vs.* $k_{obs}\,a_H$ (Fig. 1), we may determine K'_{app} (slope = $-1/K'_{app}$) and k_1 (intercept).

For the *p*-Cl esters of II and III plots of k_{obs} *vs.* *p*H possessed the shape of theoretical dissociation curves from which the pK'_{app} values of the dimethylamino group could be obtained (Fig. 2). Replotting the data for the *p*-Cl esters of II and III in the form of k_{obs} *vs.* $K'_{app}/(K'_{app} + a_H)$ provided the k_1 values as the slopes.

The values of k_{obs} determined at *p*H 8.0 (for the *p*-NO_2 esters the value of k_{obs} at *p*H 8.0 was extrapolated

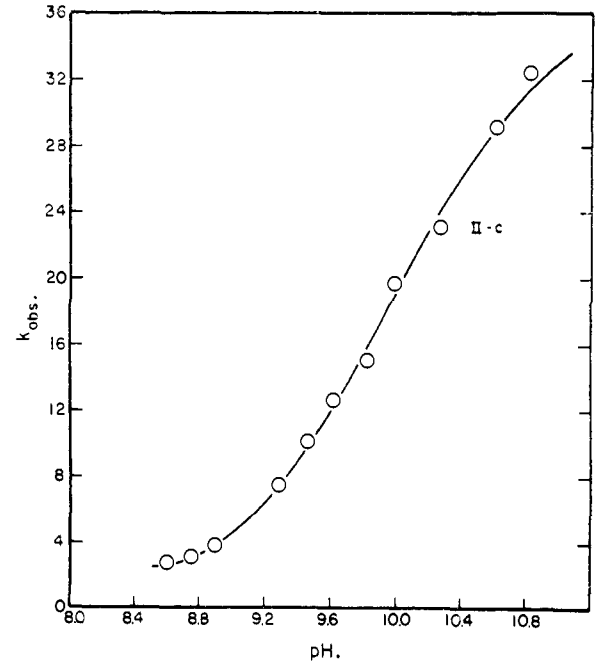


Fig. 2.—Partial *p*H–rate profile for the intramolecular nucleophilic catalysis of *p*-chlorophenyl γ-(N,N-dimethylamino)-butyrate in water (μ = 1.0) at 20°.

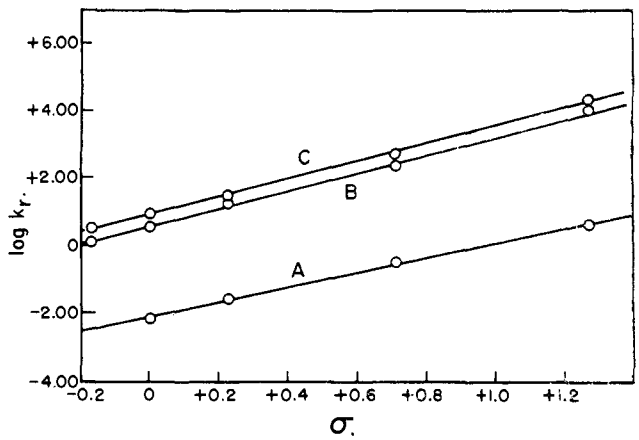


Fig. 3.—Hammett plot of the log of the true nucleophilic catalysis constant *vs.* σ for the bimolecular catalysis of substituted phenyl acylates by trimethylamine (A), for the intramolecular catalysis of substituted phenyl γ-(N,N-dimethylamino)-valerates (B) and for the intramolecular catalysis of substituted phenyl δ-(N,N-dimethylamino)-butyrates (C) in water at 20°.

from plots of log k_{obs} *vs.* *p*H when plotted in the Hammett manner (*i.e.*, log k_{obs} *vs.* σ)) provided linear plots of ρ = + 2.5 for both II and III. With the assumption that the ρ values for k_1 would also be + 2.5 a plot of log k_1 *vs.* σ was made using the determined values of k_1 for the *p*-CH_3, phenyl and *p*-chlorophenyl esters of II and III (for the limited series ρ did equal + 2.5) (Fig. 3). The values of k_1 for the *m*-NO_2 and *p*-NO_2 esters of II and III were then extrapolated from the plots. Having k_1 for the nitro esters the pK'_{app} for these esters was then obtained from the expression 2 where $k_0 = 0$. The values of pK'_{app} and k_1 so obtained at 20° are recorded in Table II.

From the data of Table II one may calculate the ratio of k_1^{II}/k_1^{III} for the similarly substituted esters in the series II and III (Table III).

It may be noted that the average pK'_{app} for the

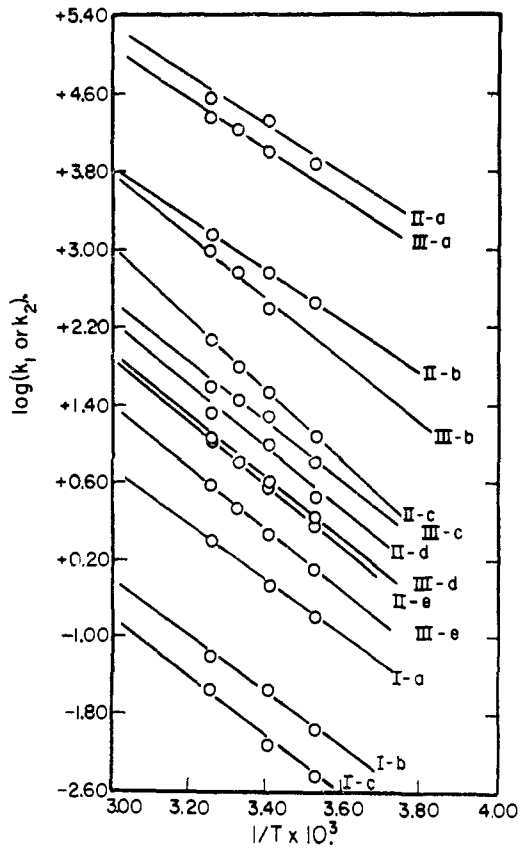


Fig. 4.—Plots of log (k_1 or k_2), the true nucleophilic catalysis constants, for I, II and III *vs.* $1/T$.

phenyl esters of II is 9.71 ± 0.12 while the average pK'_{app} for the esters of III is 9.99 ± 0.08. The slightly greater basicity of the esters of III as compared to those of II (0.28 pKa unit) is exactly that expected.[23]

In order to determine the true activation parameters it was essential to correct the rate constants determined at the various temperatures for the sizable heat of ionization (ΔH_i) of the dimethylamino group. The value of ΔH_i was determined in the following manner. The pK'_{app} values for the phenyl ester of III and the *p*-cresol ester of II were determined from *p*H–rate profiles at 10°, 20° and 34°. The apparent ΔH_i values were then determined from pK'_{app} *vs.* $1/T$ plots and were found to be 8.1 kcal. for II and 7.4 kcal. for III. These values were then reasonably assumed to hold for all esters of II and III, respectively. The pK'_{app} at any temperature (0° to 34°) could then be calculated for each ester of II or III. In turn k_1 at each temperature was then calculated from the expression $k_1 = k_{obs}(K'_{app} + a_H)/K'_{app}$. The values of k_{obs} employed to determine the true k_1 constants were determined at 10°, 20°, 27° and 34° (Fig. 4). The determined activation parameters are included in Table IV.

Bimolecular.—The rate of appearance of phenolate ion in the bimolecular reaction of trimethylamine with substituted O-acetyl phenols follows the expression

$$\text{d phenol}/dt = k_2 C_{E_T} C_{Me_3N} + k_{OH} C_{E_T} K_w/a_H \qquad (4)$$

At any constant *p*H in the presence of excess amine the determined pseudo-first-order rate constant is given by 5.

$$k_{obs} = k_2 C_{Me_3N} + k_{OH} K_w/a_H \qquad (5)$$

Therefore, the slope of a plot of k_{obs} *vs.* the concentration of free amine (*i.e.*, $A_T K_a'/(K_a' + a_H)$, where $A_T = (C_{Me_3N} + C_{Me_3NH^\oplus})$ yields the true second-order rate constant (k_2) and as intercept the values of $k_{OH}K_w/a_H = k_{OH}C_{OH^\ominus}$. The values of k_2 determined in this manner (20°) are recorded in Table V.

(23) E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Peptides," Reinhold Publishing Corp., New York, N. Y., 1943, p. 122.

TABLE II

APPARENT DISSOCIATION CONSTANTS (pK_{app}') AND FIRST-ORDER RATE CONSTANTS (k_1) FOR THE INTRAMOLECULAR NUCLEOPHILIC ATTACK OF THE DIMETHYLAMINO GROUP OF II AND III AT THE ESTER BOND (20°)

Series	Substituent	pK'_{app}	k_1, min.$^{-1}$
II	*p*-NO_2	9.60	21,500
	m-NO_2	9.82	580
	p-Cl	9.88	33.1
	H	9.70	10.0
	p-CH_3	9.53	3.50
III	*p*-NO_2	9.90	10,000
	m-NO_2	10.07	255
	p-Cl	10.13	19.9
	H	10.00	3.90
	p-CH_3	9.87	1.28

TABLE III

RATIOS OF THE RATE CONSTANTS FOR THE RING CLOSURE REACTIONS IN THE ESTERS OF II AND III (20°)

Substituent	k_1^{II}/k_1^{III}
p-NO_2	2.15
m-NO_2	2.27
p-Cl	1.67
H	2.57
p-CH_3	2.73

TABLE IV

ACTIVATION PARAMETERS FOR NUCLEOPHILIC DISPLACEMENT BY THE DIMETHYL AMINO GROUP IN I, II AND III (25°)[a]

Substituent	I, kcal. mole^{-1}		II, kcal. mole^{-1}		III, kcal. mole^{-1}	
	$\Delta H^\ddagger$	$T\Delta S^\ddagger$	$\Delta H^\ddagger$	$T\Delta S^\ddagger$	$\Delta H^\ddagger$	$T\Delta S^\ddagger$
p-NO_2	12.3	−6.3	11 9	−1.9	11.5	−2.6
m-NO_2	12.1	−8.0	11.5	−4.3	11.8	−4 4
p-Cl	12.5	−9.1	15.9	−2.2	13.8	−4.1
H	12.9	−9.4	12.5	−5.7	12.3	−6.4
p-CH_3			13.7	−5.1	14.4	−5.5

[a] The values of E_a were determined from plots of log k_1 *vs.* $1/T$, $\Delta H^\ddagger = E_a - RT$, $-T\Delta S^\ddagger = \Delta F^\ddagger - \Delta H^\ddagger$, and $\Delta F^\ddagger = RT2.303 \log (KT/hk_r)$ (Frost and Pearson, "Kinetics and Mechanism," John Wiley and Sons, Inc., New York, N. Y., 1953, pp. 95–97) standard state used was 1 mole l.$^{-1}$

TABLE V

SECOND-ORDER RATE CONSTANTS FOR THE REACTION OF TRIMETHYLAMINE (pK_a' 10.27) WITH A SERIES OF *p*-SUBSTITUTED O-ACETYLPHENOLS (20°)

Substituent	k_2, l. mole^{-1} min.$^{-1}$
p-NO_2	4.29
m-NO_2	0.342
p-Cl	.0308
H	.00795

The value of k_2 for *p*-cresol acetate could not be determined above that for k_{OH}. The values of k_2 were determined for each ester at 10°, 20° and 34° and were corrected in each case for the ΔH_i of trimethylamine (determined under our conditions to be 9.3 kcal.). From the various rate constants $\Delta H^\ddagger$ and $T\Delta S^\ddagger$ (25°) were determined and are included in Table IV.

A plot of log k_2 *vs.* σ was linear with a slope (ρ) of +2.2 (Fig. 3).

In the collection of data necessary to evaluate $\Delta F^\ddagger$, $\Delta S^\ddagger$, $\Delta H^\ddagger$ and ρ for system I there was obtained the necessary data to determine these parameters for $OH^\ominus$-catalyzed hydrolysis of substituted O-acetylphenols. Apparent k_{OH}' constants (5) were obtained from the intercepts of the plots of k_{obs} *vs.* C_{Me_3N} and related to

TABLE VI

ACTIVATION PARAMETERS FOR THE HYDROXIDE ION-CATALYZED HYDROLYSIS OF SUBSTITUTED O-ACETYLPHENOLS

Substituent	$\Delta H^{\ddagger}$, kcal. mole^{-1}	$T\Delta S^{\ddagger}$ (25°)
p-NO_2	10.1	−5.3
m-NO_2	10.3	−5.5
p-Cl	9.4	−7.0
H	10.1	−6.8

TABLE VII

RATIO OF INTRAMOLECULAR:INTERMOLECULAR CATALYTIC RATE CONSTANTS FOR THE HYDROLYSIS OF m- AND p-SUBSTITUTED O-ACETYLPHENOLS

Nucleophile / Substituent	$(CH_3)_2N^-$[a] M,	$C_3H_2N_2$[b] min.$^{-1}$/l. mole^{-1} min.$^{-1}$
H	1260	24
p-Cl	1080	23
m-NO_2	1700	32
p-NO_2	5370	9.4

[a] Intramolecular reaction, γ-(N,N-dimethylamino)-butyrates. Intramolecular reaction, γ-(4-imidazolyl)-butyrates.

the true bimolecular constant (k_{OH}) for hydroxide ion catalysis by the expression

$$k_{OH} = k_{OH'}\,(a_H/K_w) \qquad (6)$$

The temperature dependence of K_w was considered in our calculations. Plots of log k_{OH} *vs.* $1/T$ lead to the values of $\Delta H^{\ddagger}$ and $T\Delta S^{\ddagger}$ which are summarized in Table VI. The ρ-value at 30° was found to be +1.1 in agreement with previous studies.[17]

Discussion

Various attempts have been made to determine quantitatively just what type of acceleration might be obtained in converting an intermolecular nucleophilic catalysis to an intramolecular nucleophilic catalysis and to see if the mechanisms for both processes are similar. Until the present none have been completely satisfactory. The earlier comparisons have been summarized by Bender.[3] Three sets of data now appear to be worthy of consideration. All three involve a comparison of bimolecular and intramolecular nucleophilic catalysis of the hydrolysis of a series of m- and p-substituted phenyl esters. In chronological order, the first involves the comparison of the bimolecular catalysis of the hydrolysis of the phenyl esters by imidazole to the intramolecular catalysis of the hydrolysis of phenyl esters of γ-(4-imidazolyl)-butyric acid.[7] In this study activation parameters were not determined. A rough approximation of efficiency may be obtained, however, by dividing the first-order rate constant for the intramolecular reaction by the second-order rate constant for its bimolecular counterpart in Table VII. In so doing, a number is obtained in units of moles l.$^{-1}$ (*i.e.*, time^{-1}/l. moles^{-1} time^{-1} = M), and one may suppose that this would be the hypothetical concentration at which the catalyst, in the bimolecular reaction, would have to be raised in order to obtain a pseudo-first-order rate constant equivalent to the true rate constant for the intramolecular reaction. Of course these concentrations are not obtainable, but the method serves to show the very large enhancements obtained and is equivalent to the comparison of $\Delta F^{\ddagger}$ values.

In the second set Gaetjens and Morawetz[24] studied the intramolecular catalysis of the hydrolysis of substituted monophenyl esters of glutaric and succinic acids (Table VIII). They obtained a limited number of activation parameters for the intramolecular reaction and were able to compare these to a smaller number of activation terms for the bimolecular catalysis of the hydrolysis of substituted phenyl acetates by acetate ion. The bimolecular reactions were studied in the range of 60° and the intramolecular reactions in the range of 25°. With this in mind, it may be noted that the intramolecular displacement reaction is much more sensitive to the effects of strongly electron-withdrawing p-substituents than the analogous bimolecular reactions—a ρ = +2.5 for the former compared to "ρ" = +1.1 for the latter. In the comparison of the rates of hydrolysis of γ-(4-imidazolyl)-butyrate esters with imidazole participation to the bimolecular hydrolysis of phenyl acetates by imidazole ρ changes from, +1.7 to +1.35 with the bimolecular hydrolysis being more sensitive. In the present study the intramolecular catalysis for both the γ-(N,N-dimethylamino)-butyrate and δ-(N,N-dimethylamino)-valerates is more sensitive to electron withdrawing substituents than the corresponding bimolecular hydrolysis of phenyl acetates as catalyzed by trimethylamine, the ρ values changing from +2.5 to +2.2. The small changes in ρ obtained on converting the bimolecular displacements involving imidazole and trimethylamine to their intramolecular counterparts most certainly do not indicate changes in mechanism. However, the large change in ρ accompanying the conversion of the bimolecular catalysis by acetate anion to its intramolecular counterpart definitely does suggest a change in mechanism.

TABLE VIII

ACTIVATION TERMS FOR INTER- AND INTRAMOLECULAR CATALYSIS OF HYDROLYSIS OF PHENYL ACYLATES BY CARBOXYL ANION[24]

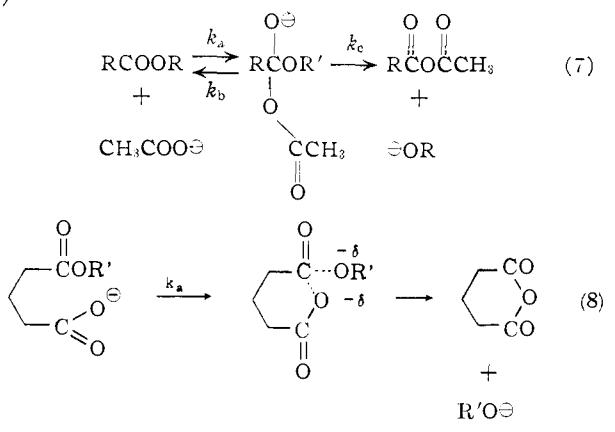
		Aco⊖	Catalysis		Monophenyl glutarates			Monophenyl succinates		
			$\Delta H^{\ddagger}$	$T\Delta S^{\ddagger}$		$\Delta H^{\ddagger}$	$T\Delta S^{\ddagger}$		$\Delta H^{\ddagger}$	$T\Delta S^{\ddagger}$
		k_2, M. sec.$^{-1}$			k_1, sec.$^{-1}$	Kcal./mole		k_1, sec.$^{-1}$	Kcal./mole	
Substituent		× 10^4 (60°)	Kcal./mole		× 10^4 (25°)		(25°)	× 10^4 (25°)		(25°)
p-OCH_3					0.041	19.3	−5.5	6.2	19.0	−2.8
p-CH_3					.048	19.5	−5.2			
H		0.102	16.6	−9.3	.098					
p-Cl					.37	20.3	−3.2			
p-$COOCH_3$					4.8					
p-NO_2		1.5	15.7	−8.5	53	19.1	−1.8			

In addition, the decided dependence of rate on $\Delta S^{\ddagger}$ in the intramolecular reactions as compared to the bimolecular reaction as catalyzed by carboxyl ion (Table VIII) further substantiates a definite difference in mechanism for the two processes. Substituent effects on the ionization constants of phenol are related, in the main, to changes in ΔS but not to ΔH of ionization[25,26]

Substituent	ΔH	$T\Delta S$ (25°)	Ref.
p-NO_2	4.70	−5.00	25
m-NO_2	4.70	−6.70	25
p-Cl	5.80	−7.00	25
H	5.65	−8.00	25, 26
p-CH_3	5.52	−8.25	26

(24) E. Gaetjens and H. Morawetz, *J. Am. Chem. Soc.*, **82**, 5328 (1960).
(25) L. P. Fernandez and L. G. Hepler, *ibid.*, **81**, 1783 (1959).
(26) D. T. Y. Chen and K. J. Laidler, *Trans. Faraday Soc.*, **58**, 4809 (162).

The marked parallel dependence of the intramolecular catalytic coefficients upon $\Delta S^{\ddagger}$ suggests that phenoxide ion is being formed in the critical transition state. In addition, the rates for intramolecular carboxyl group participation in the succinate monophenyl esters far exceed those for the glutarate series. This may be interpreted on the basis that the attack of the carboxyl ion on the ester bond is also important in the critical transition state. On the basis of this evidence (*i.e.*, ρ values, influence of ring size, $\Delta S^{\ddagger}$ values), Morawetz[24] postulates that the normal addition elimination mechanism expected for the bimolecular reaction 7 is supplanted in the intramolecular reaction by an S$_N$2 displacement not involving a tetrahedral intermediate (8)



(7)

(8)

In 8 it is certain that anhydride is formed as an intermediate. Thus, Bruice and Pandit[11] were able to identify maleic anhydride and 3,6-endoxo-Δ^4-tetrahydrophthalic anhydride as products of the solvolysis of their monophenyl esters. It now appears certain that a change of mechanism does occur in the conversion of the acetate anion-catalyzed hydrolysis of phenyl acetates to the intramolecular counterpart but that the change of mechanism is from general base in the bimolecular to nucleophilic catalysis in the intramolecular reactions. Thus Butler and Gold[27] have recently established that the reaction of acetate anion with *p*-nitrophenyl acetate does not, in the main, go by way of acetic anhydride as an intermediate. The latter reaction appears to proceed primarily *via* general base catalysis. Most likely, then, the catalysis of the hydrolysis of phenyl acetate by acetate anion is strictly of the general base type. The latter conclusion arises from considerations of the relative sensitivity of *p*-nitro and phenyl acetate to general base-catalyzed ammonolysis.[17] This change of mechanism (*i.e.*, general base-catalyzed hydrolysis to nucleophilic catalysis) occurring on conversion of a bimolecular catalysis to its intramolecular counterpart is of profound interest and is receiving further study in this Laboratory.

The intramolecular nucleophilic catalysis by carboxyl anion, as studied by Morawetz, shares in common with the bimolecular and intramolecular systems of this study (I, II, III and $OH^{\ominus}$ catalysis) the fact that $\Delta H^{\ddagger}$ is independent of the nature of the substituent group and that $T\Delta S^{\ddagger}$ is dependent on the nature of the substituent group. The values of $\Delta H^{\ddagger}$ for these reactions is dependent only on the nature of the nucleophile (*i.e.*, R–N$(CH_3)_2$ *vs.* $OH^{\ominus}$) but is independent of whether the reaction is of an intramolecular (II and III) or intermolecular (I) type. Except in the case of the *p*-chloro esters, the change in $\Delta H^{\ddagger}$ accompanying the conversion of I → II or I → III amounts to less than a kcal./mole.

(27) A. R. Butler and V. Gold, *J. Chem. Soc.*, 1334 (1962).

	$\Delta\Delta H^{\ddagger}$, kcal. mole^{-1}	
	I–II	I–III
p-NO_2	0.4	0.8
m-NO_2	0.6	0.3
p-Cl	−3.4	−1.3
H	0.4	0.6

Comparing the values of $T\Delta S^{\ddagger}$ for I to those for II and III shows that the formation of the five-membered ring of II is favored by *ca.* 4.7 kcal. mole^{-1} (~16 e.u. at 25°) and the formation of the six-membered ring of III is favored by *ca.* 3.8 kcal. mole^{-1} (~13 e.u. at 25°) over the bimolecular reaction of I.

	$\Delta T\Delta S^{\ddagger}$, kcal. mole^{-1}	
	I–II	I–III
p-NO_2	−4.4	−3.7
m-NO_2	−3.7	−3.6
p-Cl	−6.9	−5.0
H	−3.7	−3.0
	−4.7 ± 1.1	−3.8 ± 0.6

The increase in efficiency of II and III with respect to I (Table VII) must reside in the gain in translational entropy brought about by bringing the reactant groups together, there being no evidence for any change in mechanism.[28] Clearly then, all steric and electronic influences on $\Delta F^{\ddagger}$, in the systems I, II, III, as well as in the case of OH^--catalyzed hydrolysis of O-acetylphenols and in the solvolysis of the monophenyl esters of succinic and glutaric acids, are essentially apparent only in changes of $\Delta S^{\ddagger}$. The alterations in $\Delta S^{\ddagger}$ on substitution are more apparent in I, II, III and in the monophenyl esters of the dibasic acids than in the case of OH^- catalysis. This is as expected on the basis that the ρ values for the former are at least twice that of the latter.

There remains to be explained just why, in the systems discussed above, the electronic effect of substituent groups alters $\Delta S^{\ddagger}$ and not $\Delta H^{\ddagger}$. As far as the authors are aware this is a rarity in kinetics,[29] though the dissociation constants of oxyacids in general are related primarily to alterations in ΔS.[30]

Whether electronic effects on $\Delta F^{\ddagger}$ are reflected in alterations of $\Delta H^{\ddagger}$ or $\Delta S^{\ddagger}$ may be dictated by the solvent. Thus, for the alkaline hydrolysis of substituted O-acetylphenols the ρ value is always *ca.* 1.1 regardless of solvent (H_2O, ρ = 1.1 (this study and ref. 23); 28.5% EtOH–H_2O(v./v.), ρ = 1.15[17]; 60% acetone–water (st.%), ρ = 1.15[32]) though the values of ΔF are greatly solvent dependent. Of greatest interest is the fact that electronic effects show up in $\Delta S^{\ddagger}$ in aqueous solution of μ = 1.5 (this study) but show up in $\Delta H^{\ddagger}$ in 60% acetone water[31]:

	Kcal. mole^{-1}	
	$\Delta H^{\ddagger}$	$T\Delta S^{\ddagger}$ (25°)
H	11.9	5.7
m-CH_3	12.3	5.6
p-CH_3	12.3	5.7
m-NO_2	10.7	5.6
p-NO_2	10.4	5.7

It has not been ascertained if the systems I, II, III as well as the monophenyl esters of glutaric acid and succinic acid might behave similarly. A working hypothesis assumes a mechanism proceeding through a

(28) M. L. Bender, E. J. Pollock and M. C. Neveu, *J. Am. Chem. Soc.*, **84**, 595 (1962), have recently shown the reaction of trimethylamine with phenyl acetate does not exhibit a D_2O solvent isotope effect.

(29) L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., Chapt. VII, 1940.

(30) L. Eberson and I. Wadso, *Acta Chem. Scand.*, in press.

(31) Extrapolated from the data of E. Tommila and C. N. Hinshelwood, *J. Chem. Soc.*, 1801 (1938).

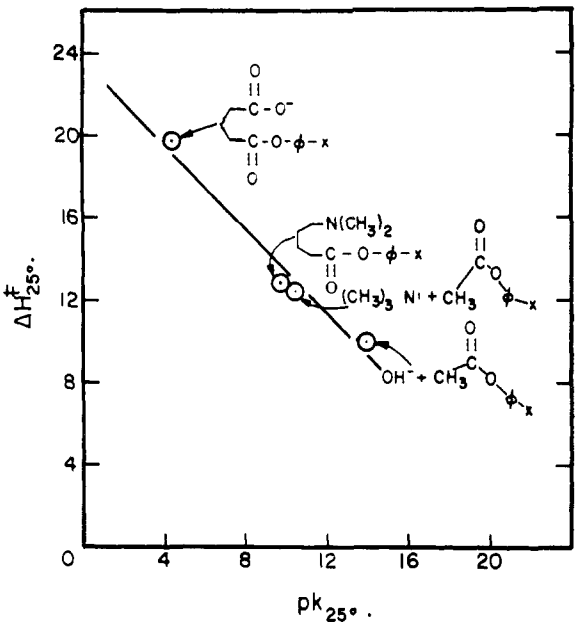
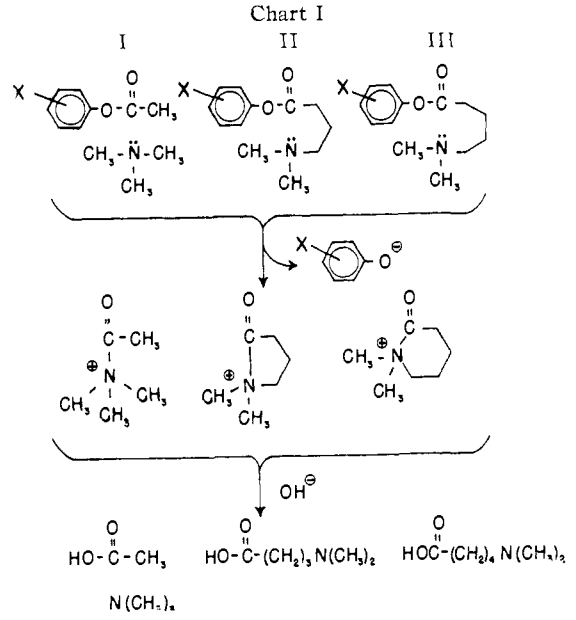


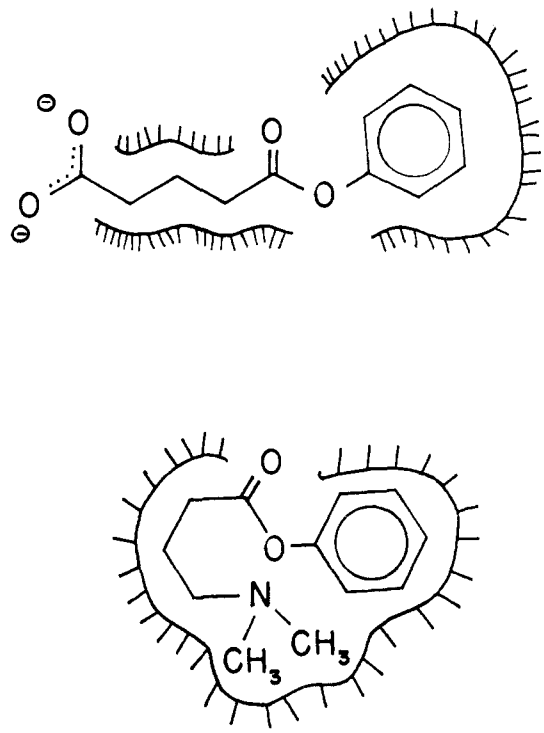
Fig. 5.—Plot of $\Delta H^{\ddagger}$ *vs.* $pK_{25}°$ for those nucleophiles whose catalysis of *o*-substituted phenyl acylates is characterized by a constant $\Delta H\ddagger$.

Chart I



where X = a, *p*-NO_2; b, *m*-NO_2; c, *p*-Cl; d, H; e. *p*-CH_3

Chart II



tetrahedral intermediate (7) in which the free energy of the transition states associated with the formation of the bond between the nucleophile and the carbonyl carbon as well as that for the departure of the leaving group were comparable to that for the tetrahedral intermediate.[32] Electronic effects on the formation of the bond between the nucleophile and the carbonyl group would be expected to be evident in $\Delta H^{\ddagger}$ while the departure of the leaving phenoxide ion would be expected to be most evident in $\Delta S^{\ddagger}$. For such a mechanism the solvent could determine if the formation of the bond between the nucleophile and ester or the departure of the phenoxide ion from the tetrahedral intermediate were of the greatest importance, and thus determine the effect of substituent groups on $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$. The importance of ring size on the magnitude of k_a has been considered[9,11] and for a mechanism of this type $k_{rate} = k_a(k_b/(k_b+k_c))$ where neither k_b nor k_c can be ignored; ring size would always be important regardless of whether the k_a or k_c term were of greatest significance (7).[33]

It has previously been noted[34] that the Brönsted equation (*i.e.*, log $k_2 = \alpha pK_a' + C$) adequately correlated the rate constants for displacement of *p*-nitrophenol from O-acetyl-*p*-nitrophenol when bases of the same type were employed (*i.e.*, 4(5)-substituted imidazoles) but that different plots were obtained if the type base was changed (imidazoles, anilines, pyridines, phenoxide ions, etc.). The present study suggests that a more meaningful plot might be that of 11.

$$\Delta H^{\ddagger} = \alpha pK_a + C \qquad (11)$$

A plot of this type is presented in Fig. 5. It may be noted that bases widely divergent in type fit one single line. Whether this is fortuitous or not awaits further investigation. It may be noted that the values of $\Delta H^{\ddagger}$ for imidazole[17,35] and thiol anion[36] attack on O-acetyl-*p*-nitrophenol exhibit negative deviations from the plot of Fig. 5 (10 and 8 kcal. mole^{-1}, respectively); however, it is not yet known whether these bases operate *via* the same type mechanism (*i.e.*, constant $\Delta H^{\ddagger}$ and varying $\Delta S^{\ddagger}$ with substitution) in H_2O.

The ratio of the rate constants for the formation of the five-membered ring to that for the formation of the six-membered ring (*i.e.*, II *vs.* III) were found to be 2.3 ± 0.3; Table III. This value is just one-hundredth the ratio obtained for the solvolysis of the glutarate

(32) Such a mechanism would be experimentally indistinguishable from a direct S$_N$2 displacement reaction. It would be in agreement with, but not uniquely required to explain, the known fact that in the alkaline hydrolysis of phenyl benzoates in H_2O^{18} no O^{18} is back incorporated into the ester (C. A. Bunton and D. N. Spatcher, *J. Chem. Soc.*, 1079 (1956)), since in such a mechanism the values of k_2 and k_3 would be greater than the rate of proton exchange (M. Eigen, *Disc. Faraday Soc.*, **17**, 204 (1954)). Evidence has recently been presented that the rates of proton exchange may be kinetically significant in the alkaline hydrolysis of *p*-substituted methyl benzoates (M. L. Bender and R. T. Thomas, *J. Am. Chem. Soc.*, **83**, 4189 (1961)).

(33) Alternatively if k_b became very much greater than k_c, the importance of ring size might be determined by the relative stability of the carbonyl double bond in the product (H. C. Brown, *J. Org. Chem.*, **22**, 439 (1957)).

(34) T. C. Bruice and R. Lapinski, *ibid.*, **80**, 2265 (1958).

(35) M. L. Bender and B. W. Turnquest, *ibid.*, **79**, 1652 (1957).

(36) J. R. Whitaker, *ibid.*, **84**, 1900 (1962).

and succinate esters.[9,11] A possible explanation for this difference could reside in the preferential conformation of the ground states for the dimethylamines and the carboxylic acids. Possibly owing to the solvation of the carboxyl anion the glutarate and succinate monoesters exist preferentially in an extended conformation, whereas the relatively non-polar amines exist in a coiled conformation due to formation of hydrophobic bonds (Chart II). In this connection it should be noted that rate studies conducted in 50% dioxane–water and also in 8 *M* aqueous urea solution failed to alter this ratio.[37]

(37) These experiments are probably not critical since the uncoiling of hydrocarbon chains due to clathrate formation requires at least a 7-membered chain (see L. F. Fieser and M. Fieser, "Advanced Organic Chemistry," Reinhold Publishing Corp., New York, N. Y., 1961, pp. 131–133). Also, the water content of 50% dioxane–water would probably not be low enough to bring about uncoiling.

A second explanation would implicate steric hindrance imposed by the dimethyl substitution of the amino group on the rate of closure to form the five-membered ring (though the magnitude of the rate constants would not appear to suggest any steric hindrance; see Table VII). It should be noted that the ratio of the rate constants for the formation of five- and six-membered rings in the cyclization of ω-aminoalkyl bromides in water is 800.[38] Our investigations in this area are continuing.

Acknowledgments.—This work was supported by grants from the National Science Foundation and the National Institutes of Health. S. J. Benkovic particularly acknowledges support in the nature of a predoctoral fellowship from the National Institutes of Health.

(38) G. Solomon, *Helv. Chim. Acta*, **16**, 1361 (1933); **17**, 851 (1934).

[CONTRIBUTION FROM THE CHEMISTRY DIVISION, ARGONNE NATIONAL LABORATORY, ARGONNE, ILL.]

Isolation, Amino Acid Composition and Some Physico-chemical Properties of the Protein Deuterio-phycocyanin[1]

BY DONALD S. BERNS,[2] HENRY L. CRESPI AND JOSEPH J. KATZ

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The isolation and purification of a fully deuteriated protein, deuterio-phycocyanin, from blue-green algae grown autotrophically in 99.8% D_2O is described. Sedimentation behavior in the ultracentrifuge shows the protein to be a system of reversibly interacting components. The amino acid compositions of ordinary and deuterio-phycocyanin isolated from *Plectonema calothricoides* have been established and it appears that the amino acid compositions are identical within experimental error. Ordinary and deuterio-phycocyanin therefore probably differ only in isotopic composition. Thermal denaturation of ordinary and deuterio-phycocyanin, both dissolved in H_2O, has been studied by measuring the quenching of fluorescence. Deuterio-phycocyanin undergoes thermal denaturation at a temperature 5° lower than is the case for ordinary phycocyanin. Since the two proteins appear to differ primarily in the isotopic composition of the non-polar side chains, differences in denaturation behavior are probably to be ascribed to differences in hydrophobic bonding.

Introduction

The successful cultivation of fully deuteriated organisms on the large scale by Katz, Crespi and coworkers[3] has made a great variety of fully deuteriated substances accessible for the first time. Proteins in which hydrogen has been entirely replaced by deuterium may be expected to make a useful contribution to problems of protein structure and function, and efforts have therefore been directed to the preparation of such substances. In the present communication the isolation, purification, amino acid composition and behavior on thermal denaturation of the fully deuteriated protein phycocyanin, extracted from the fully deuteriated blue-green alga *Plectonema calothricoides*, are described; a preliminary description of some aspects of this work has already appeared.[4]

Phycocyanin is a blue photosynthetic pigment widely distributed in blue-green algae. A member of the class of biliproteins, for which molecular weights of the order of 200,000 to 300,000 have been quoted, phycocyanin appears in fact to be a system of reversibly interacting components. The chemical properties of phycocyanin are reviewed by O'hEocha.[5] For purposes of the present discussion the ordinary, hydrogen-containing phycocyanin extracted from algae grown in H_2O will be referred to as ordinary or protio-phycocyanin; the protein extracted from algae grown in 99.8% D_2O will be designated deuterio-phycocyanin. The prefix deuterio- will also be used when the deuterio-phycocyanin is dissolved in H_2O and the exchangeable positions of the protein are occupied by hydrogen. The sharp distinction between the protein described here, in which the non-exchangeable positions in the molecule are occupied by deuterium, and ordinary proteins into which deuterium is introduced into exchangeable positions by treatment with D_2O[6] can be readily inferred from the discussion of Scheraga[7] on hydrogen–deuterium exchanges in proteins.

Experimental

Isolation and Purification.—Deuterio-phycocyanin and protio-phycocyanin were isolated from the blue-green alga *Plectonema calothricoides* by either of two procedures. In the first, approximately 25 g. of algae (wet weight) were frozen and thawed twice to rupture the cells. About 200 ml. of aqueous acetate buffer (pH 4.7, μ = 0.1) was added and the solution allowed to stand, first for several hours at room temperature, and then in the refrigerator at 5°. After several days, the supernatant solution was quite blue and intensely fluorescent. The supernatant solution was removed by centrifugation, fresh buffer was added, and the extraction continued until most of the phycocyanin was removed from the algae. An olive-green appearance of the residue indicated that most of the blue pigment had been extracted. The aqueous extract was then centrifuged for 10 minutes at 4° at 12,000 r.p.m. to remove debris. Phycocyanin was then precipitated from the clarified extract by adding ammonium sulfate to 50% saturation. The second procedure for the extraction of phycocyanin from algae omitted freezing and thawing. Instead, cell lysis was achieved by the action of lysozyme (Worthington 2× crystallized, 10 μg. per ml.) in phosphate buffer (pH 7, μ =

(1) Based on work performed under the auspices of the U. S. Atomic Energy Commission. Presented in part at the 141st National Meeting of the American Chemical Society in Washington, D. C., April, 1962.

(2) Resident research associate, 1961–1962.

(3) H. DaBoll, H. L. Crespi and J. J. Katz, *Biotechnology and Bioengineering*, to be published.

(4) D. S. Berns, H. L. Crespi and J. J. Katz, *J. Am. Chem. Soc.*, **84**, 496 (1962).

(5) C. O'hEocha, in "Comparative Biochemistry of Photoreactive Systems," M. B. Allen, ed., Academic Press, Inc., New York, N. Y., 1960, pp. 181–205.

(6) L. G. Augenstine, C. A. Ghiron, K. L. Grist and R. Mason, *Proc. Natl. Acad. Sci.*, **47**, 1733 (1961); J. Bello and D. Harker, *Nature*, **192**, 756 (1961).

(7) H. A. Scheraga, "Protein Structure," Academic Press, Inc., New York, N. Y., 1961, pp. 192–208.