more unequivocally than in ultraviolet spectroscopy. Raman spectra require much higher concentrations of solute than infrared spectra for accurate measurement; on the other hand, Raman spectra are well adapted for aqueous solutions, since the Raman bands of water appear in only two limited regions, and the bands due to the solute appear clearly everywhere else. The sulfhydryl group, which we have studied in this paper, is particularly suitable for such study, since the S-H stretching frequency at 2580 is a very strong line in the Raman spectrum, although relatively weak in the infrared.

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Hydroxyl Group Catalysis. II. The Reactivity of the Hydroxyl Group of Serine. The Nucleophilicity of Alcohols and the Ease of Hydrolysis of Their Acetyl Esters as Related to Their pKa'*

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Since the hydroxyl group of peptide-bound serine is purported to be the nucleophilic center of many esteratic enzymes, it is important to ascertain if this group possesses special nucleophilic properties toward the ester bond. We have shown that in a suitable model, *N*-acetylserinamide, it does not. The second-order rate constant (k_T) for the reaction of $ArO^{\ominus}$ and $AlkO^{\ominus}$ with *p*-nitrophenyl acetate and the second-order rate constants (k_{OH}) for the alkaline hydrolysis of $ArOCOCH_3$ and $AlkOCOCH_3$ can be correlated with the pKa' of ArOH or AlkOH *via* the expressions $\log k_T = 0.76\ pKa' - 6.3$ and $\log k_{OH} = -0.26\ pKa' + 2.56$. The pKa' of *N*-acetylserinamide has been determined conductometrically (13.60 ± 0.05) and has been found to correlate the $\log k_T$ and the $\log k_{OH}$ values for *N*-acetylserinamide anion and *N,O*-diacetylserinamide respectively. To dispel misconceptions on the mechanism of the reaction of *N*-acetylserinamide anion with *p*-nitrophenyl acetate we have determined the second-order rate constant for the reaction of $CF_3CH_2O^{\ominus}$ with *p*-nitrophenyl acetate.

In the sequence (1) both $\log k_T$ and $\log k_{OH}$ should be linear functions of the pKa' of ROH. Thus, the log of the second-order rate constants for the displacement of *p*-nitrophenol from *p*-nitrophenyl acetate by a series of 4(5)-substituted imidazoles was shown to be a linear function of the pKa' of the imidazoles employed (Bruice and Lapinski, 1958). Inferences are that this would be

$$\mathrm{ROH} \overset{Ka'}{\rightleftharpoons} \mathrm{RO^{\ominus} + H^{+}}$$

$$\mathrm{RO^{\ominus} + CH_3\overset{\overset{O}{\|}}{C}{-}OR'} \xrightarrow{k_T} \mathrm{CH_3{-}\overset{\overset{O}{\|}}{C}{-}OR + \overset{\ominus}{O}R'} \quad (1)$$

$$\mathrm{CH_3{-}\overset{\overset{O}{\|}}{C}{-}OR} \xrightarrow[OH^{\ominus}]{k_{OH}} \mathrm{CH_3COO + R\overset{\ominus}{O}}$$

so also for other series of nucleophiles, including aryl and alkoxides (Bruice and Lapinski, 1958; Jencks and Carrioulo, 1960). The correlation of $\log k_{OH}$ to the pKa' of ROH is self-evident in the success of the Hammett $\rho\sigma$ relationship (Hammett, 1940) in correlating both pKa' of ROH and k_{OH} (Ballinger and Long, 1960; Taft, 1956).

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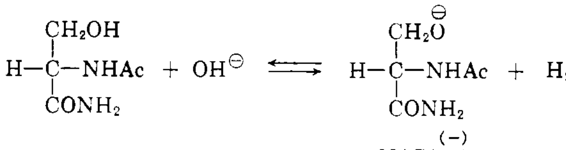
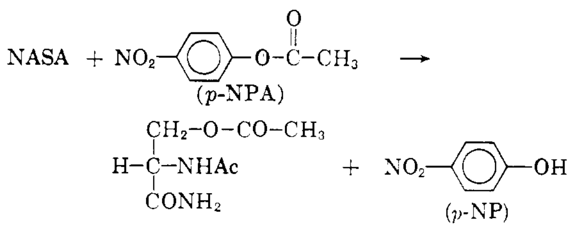
Recently Anderson, Cordes, and Jencks (1961) reported the third-order velocity constant for the hydroxyl ion–catalyzed reaction of *N*-acetylserinamide with *p*-nitrophenyl acetate (25°; H_2O) to



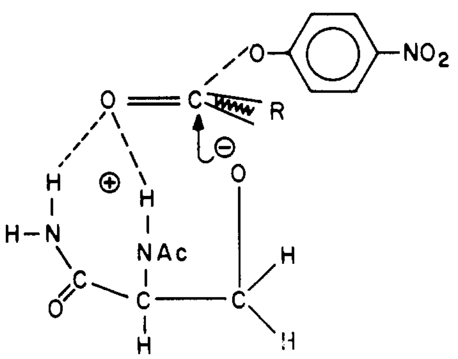
(2)



be 2.2×10^4 liter^2 mole^{-2} min.$^{-1}$. The value of k_T (assuming pKa′ values for *N*-acetylserinamide to be between 13 and 17) was estimated, by these workers, to be between 1,000 and 6,000 times greater than expected on the basis of a Brönsted plot (3) with the slope, α, equal to 0.8 (Bruice and Lapinski, 1958) and passing through the point for the hydroxyl ion.

$$\log k_T = \alpha pKa' + C \qquad (3)$$

It was concluded that "the abnormal reactivity of *N*-acetylserinamide. . . cannot be ascribed to an unusual acidity of its hydroxyl group." The "abnormally great" nucleophilicity of *N*-acetylserinamide was rationalized by assuming assistance, *via* general acid catalysis, from the neighboring amide functions (4).



(4)

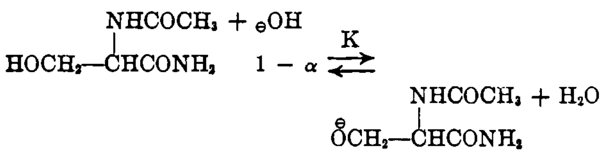
Independently (Bruice and York, 1961), it was found that the oxide anions of the polyfunctional alcohols pentaerythritol and tris-(hydroxymethyl)-aminomethane possess, in their reaction with *p*-nitrophenyl acetate, values of k_T comparable to the value of k_T associated with the reaction of *N*-acetylserinamide anion with *p*-nitrophenyl acetate. It is possible to write mechanisms for the reaction of tris-(hydroxymethyl)aminomethane and pentaerythritol with *p*-nitrophenyl acetate which involve general acid catalysis by $-CH_2OH$ groups neighboring the reactive $-CH_2O^{\ominus}$ nucleophilic centers (*i.e.*, as in the case of equation (4)). However, the arguments (Anderson, Cordes, and Jencks, 1961) supporting mechanism (4) are not compelling, being based primarily on the pKa′ of the hydroxyl ion and the comparative reactivity of the *N*-acetylserinamide anion and the hydroxyl ion toward *p*-nitrophenyl acetate. In the Brönsted relationship the nucleophilicity of the hydroxyl ion toward *p*-nitrophenyl acetate would appear to be much less than expected for other oxygen anion bases (Bruice and Lapinski, 1958). In order to understand the nucleophilicity of the oxide anions of *N*-acetylserinamide, pentaerythritol, and tris-(hydroxymethyl)aminomethane toward the ester bond of *p*-nitrophenyl acetate—and in broader terms to understand the relationship of basicity and nucleophilicity of oxygen anions toward the ester bond in general—we have determined the pKa′ of *N*-acetylserinamide and compared the values of k_T and k_{OH} (equation 1) to the corresponding values for other alcohols of known pKa′. The great interest in *N*-acetylserinamide stems from the suggestions that the serine hydroxyl group forms a portion of the active center of numerous esteratic enzymes. (For a compilation of references see Bruice and Sturtevant, 1959.)

Experimental

Compounds.—*N*-Acetylserinamide was prepared according to the procedure of Rothstein (1949) (m.p. 138–139°; reported m.p. 138–139°) and pentaerythritol monoacetate according to the procedures of Elrick and Preckel (1954) (m.p. 65–66°; reported m.p. 65–66°). The *p*-nitrophenyl acetate was prepared as in previous studies (Bruice and York, 1961) and was recrystallized from ether, m. p. 77.5–78.0°. Trifluoroethyl acetate was prepared by refluxing, for 5 hours, trifluoroethanol (15.0 g; 0.15 mole) with acetic anhydride (40.0 g; 0.39 mole) and a small amount of anhydrous $ZnCl_2$ (0.1–0.2 g). The refluxing mixture was protected with a $CaCl_2$ drying tube. The ester (15.0 g) was isolated, by fractional distillation, directly from the reaction mixture, b.p. 72-74° (740 mm), n_D^{25} 1.3190 (Henne and Pelley, 1961, give b.p. 78°, n_D^{20} 1.3202).

Apparatus.—The spectrophotometer employed was a Zeiss PMQII fitted with a thermostat-equipped brass block for holding the cuvettes. All pH measurements were made with a Radiometer Model 22 pH meter. Hydrolytic rate constants were determined with a pH-stat designed around the Radiometer T 111a autotitrator (Bruice and Fife, 1961). The conductometric apparatus has been described previously (Ballinger and Long, 1959). The conductivity cell was of conventional design with lightly platinized electrodes and had a cell constant of 0.510 cm^{-1}. The cell was maintained at 25° ± 0.005 in a bath of petroleum oil.

pKa Determination for Hydroxyl Function of N*-Acetylserinamide.*—In preliminary experiments it was found that *N*-acetylserinamide hydrolyzes slowly in dilute sodium hydroxide solution (0.00386 M). The following procedure was therefore employed: A quantity of *N*-acetylserinamide was weighed into a dry 25-ml volumetric flask. At zero time the flask was filled to the mark with 0.00386 M sodium hydroxide and shaken thoroughly. At measured intervals the resistance of the mixture was determined and plotted against time. After an initial more rapid increase the plot became linear; it was followed usually for 40 to 50 minutes. A good extrapolation could be made to the resistance at zero time. The equilibrium constant for the reaction



was calculated as described by Ballinger and Long (1959). The ionization constant for *N*-acetylserinamide is obtained by multiplication of the observed K values by the ion product for pure water (1.01×10^{-14} at 25°), since the activity coefficient correction factor $\gamma_{OR^-}\alpha_{H_2O}/\gamma_{HOR}\gamma_{OH^-}$, where γ is the activity coefficient and α is the degree of dissociation, can be approximated as unity for dilute solutions (Ballinger and Long, 1959).

$$K_a = \frac{\left[{}^{\ominus}OCH_2\overset{NHCOCH_3}{\overset{|}{C}}HCONH_2 \right]}{\left[HOCH_2\overset{NHCOCH_3}{\overset{|}{C}}HCONH_2 \right] [{}^{\ominus}OH]} \times [H^+][^-OH]$$

No correction was made for the increase in resistance of the solutions because of change in viscosity due to the added *N*-acetylserinamide, since at the concentrations employed the correction would be small and the calculated value of the equilibrium constant would not be altered to a significant extent. The effect of *N*-acetylserinamide on the resistance of a 0.005 M solution of potassium chloride is noted in Table I, the small

Table I

Equilibrium Constant for the Reaction of *N*-Acetylserinamide with Sodium Hydroxide in Water at 25°

Initial NaOH	*N*-Acetyl-serin-amide concn.	Resist-ance (ohm)		K
0.003696	0	R_0 = 573.0		
0.003696	0.0462	R = 615.0	0.0932	2.24
0.003696	0.06007	R = 629.0	0.1286	2.457
0.003865	0	R_0 = 563.0		
0.003865	0.02652	R = 585.0	0.0579	2.316
0.003865	0	R_0 = 557.0		
0.003865	0.07085	R = 624.0	0.1641	2.77
0.003865	0	R_0 = 560.7		
0.003865	0.23206	R = 719	0.3379	2.22
				2.40 Av.
(0.005 N KCl)[a]	0	R = 715		
	0.0617	R = 722		

[a] No NaOH in these experiments.

increase in resistance being due to the change in viscosity. It has been shown that alcohols produce an almost identical percentage increase in the resistance of potassium chloride and sodium hydroxide solutions, with the increase being proportional to viscosity (Ballinger and Long, 1959).

The value of λ_0 for ${}^{\ominus}OCH_2\overset{NHCOCH_3}{\overset{|}{C}}HCONH_2$, where λ is the ionic mobility of the ion at the prevailing total electrolyte concentration, c, was determined graphically to be 34 by the method described by Ballinger and Long (1959). Assuming that the value lay between 25 and 40, the difference in pK would have been 0.05 to 0.06 pK units; therefore, a small error in this constant would not significantly affect the reported pK value. The value of $\lambda_0 Na^+$ at the concentration of sodium hydroxide employed was taken to be 48.1 (calculated by Ballinger and Long, 1959, from the data of Harned and Owen, 1950).

The Ka value for *N*-acetylserinamide (*i.e.*, $K \times K_W$) is, therefore, 2.42×10^{-14}, and the pKa = 13.60 ± 0.05.

Kinetics.—Ester hydrolysis was followed titrimetrically at constant pH and the *pseudo*-first-order rate constants calculated by the method of Guggenheim (1926). The determined values of k_{OH} are included in Table II.

Table II

The pKa' of Various Alcohols, the Rates of Alkaline Hydrolysis (k_{OH}) of their Acetyl Esters, and the Rates of the Reaction of their Oxanions (k_T) with *p*-Nitrophenyl Acetate[a] (*p*-NPA)

Alcohol	pKa'	k_{OH} l. mole^{-1} min^{-1}	k_T
CH_3CH_2OH	16.00[b]	5.22[j]	
$CH_2{=}CHCH_2OH$	15.52[b]	12.5[j]	
CH_2OHCH_2OH	14.77[b]	16.3[j]	
$ClCH_2CH_2OH$	14.31[c]	17.0[k]	
$CH_2OHCHOHCH_2OH$	14.1[b]	17.2[j]	
$C(CH_2OH)_4$	14.0[b]	24.6[d]	1.26×10^4[n]
$CH{\equiv}CCH_2OH$	13.55[b]	43.8[j]	
$H_2NCO{-}CH(NHCOCH_3)CH_2OH$	13.6[d]	48.8[l]	9×10^3[o]
F_3CCH_2OH	12.36[e]	41[d]	4.7×10^3[p]
p-$CH_3C_6H_4OH$	10.19[f]	135[m]	
C_6H_5OH	9.98[g]	223[m]	19.0[i]
p-ClC_6H_4OH	9.36[g]	417[m]	9.6[i]
m-$NO_2C_6H_4OH$	8.34[g]	683[m]	
p-$NO_2C_6H_4OH$	7.14[g]	1410[m]	
p-$CHOC_6H_4OH$	7.6[h]		0.5[i]
(CH_3COO^-)	(4.7)[i]		(0.00095)[i]

[a] Where possible, data obtained at 25° in water have been collected. However, in certain cases data from rate studies carried on at 30° or in mixed aqueous-ethanol solvents have been tabulated. Neither the difference in solvent nor the 5° temperature difference had an effect great enough to invalidate the logarithmic plots for which these data are used nor to invalidate the arguments derived from such plots. [b] Ballinger and Long (1960). [c] Ballinger and Long (1959b). Solv. H_2O, T = 25°. [d] This study. Solv. H_2O, T = 25°. [e] Ballinger and Long (1959a). Solv. H_2O, T = 25°. [f] Fickling *et al.* (1959). Solv. H_2O, T = 25°. [g] Fernandez and Hepler (1959). Solv. H_2O, T = 25°. [h] Gawron *et al.* (1952). Solv. H_2O, T = 25°. [i] Bruice and Lapinski (1958). [j] Extrapolated to 25° (solvent H_2O) from the data collected in Table No. 212.441 of Supplement 1 of Tables of Chemical Kinetics, National Bureau of Standards (U.S.), Circular 510 (1956). [k] The lactate ester of 2-chloroethanol (Solv. H_2O, T = 25°) hydrolyzes 3.26 times as fast as ethyl lactate. It is tacitly assumed that the acetate ester of 2-chloroethanol also hydrolyzes 3.26 times as fast as ethyl acetate. Data collected in Table No. 212.441 of Supplement 1 of Tables of Chemical Kinetics, National Bureau of Standards (U.S.), Circular 510 (1956). [l] Anderson *et al.* (1961). Solv. H_2O, T = 25°. [m] Bruice and Mayahi (1960). Solv. H_2O, T = 30°. [n] Bruice and York (1961). [o] Calculated from the value of k_3; *i.e.*, k_3 (*N*-acetylserinamide) (*p*-nitrophenyl acetate) (hydroxyl ion) reported at 25° (Anderson *et al.*, 1961) and the pKa' of *N*-acetylserinamide as determined in this study. Solv. H_2O, T = 25°. [p] This study. Solv. H_2O, T = 30°.

Reaction of Trifluoroethanol with p-*Nitrophenyl Acetate.*

Solutions.—(A) For each pH employed a solution of 1.0 M trifluoroethanol and 0.02 M K_2CO_3 was prepared. The solution was adjusted to the desired pH with standard hydrochloric acid and to a calculated ionic strength (μ) of 1.0 M with KCl. (B) For each pH employed there was prepared a solution of 0.02 M K_2CO_3 adjusted to $\mu = 1.0$ M with KCl and adjusted to the desired pH. (C) A solution of 0.090 g of *p*-nitrophenyl acetate in 10.0 ml of anhydrous ether was prepared. When 2 drops of this solution, added from a calibrated capillary dropper, was diluted to 10 ml, a solution approximately 1×10^{-4} M in *p*-nitrophenyl acetate was obtained.

The correct volumes of solutions (A) and (B) were mixed in a 10-ml volumetric flask to give the desired molarity of trifluoroethanol. After equilibration at 30°, 2 drops of the stock solution of *p*-nitrophenyl acetate (C) was added and the reaction mixture transferred to a 4-ml quartz cuvette thermostated (30°) in the spectrophotometer. The liberation of *p*-nitrophenolate ion was followed by observing the increase in density at 400 mμ. pH determinations before addition of ester to the reaction mixture and after completion of reaction were found to be identical in every case. The *pseudo*-first-order rate constants were calculated from the expression

$$\log \frac{\text{O.D.}_\infty}{\text{O.D.}_\infty - \text{O.D.}_t} = k_{obs}t$$

Results and Discussion

Of the alcohols considered in the introduction to this study, pentaerythritol was the only one for which the pKa′ had been determined (14.0 at 25°) (Ballinger and Long, 1960). This value, as expected on the basis of the inductive effect of the four hydroxymethyl groups, is considerably smaller than that for methanol and ethanol (Ballinger and Long, 1960). We have now (see Experimental) determined the pKa′ of *N*-acetylserinamide to be 13.60 ± 0.05 at 25°. The values of k_T and pKa′ for *N*-acetylserinamide and pentaerythritol are, therefore, quite similar. Both pentaerythritol and *N*-acetylserinamide then possess pKa′ values lower than those of ordinary aliphatic alcohols. The obvious experiment to eliminate mechanism (4) as operative for *N*-acetylserinamide or pentaerythritol is to determine the value of k_T for the reaction with *p*-nitrophenyl acetate of an alcohol which does not possess acidic functional groups but does possess a pKa′ lower than that of a normal aliphatic alcohol. For this purpose we chose to investigate trifluorethanol, which has a pKa′ of 12.36 (Ballinger and Long, 1960).

In the presence of excess trifluoroethanol, and at constant pH, the rate of solvolysis (k_{obs}) of *p*-nitrophenyl acetate was found to be linearly dependent on the concentration of trifluoroethanol (Fig. 1). Furthermore, the *pseudo* catalytic coefficients for trifluoroethanol (k'_2), as determined from the slopes of the plots of Figure 1, were found to be linearly dependent on the concentration

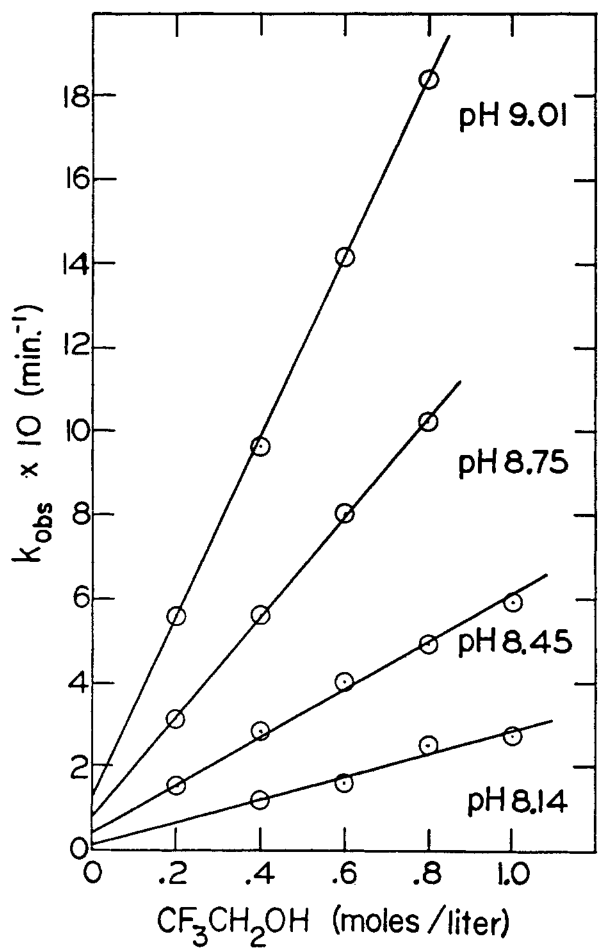


Fig. 1.—The dependence of the *pseudo*-first-order solvolysis constant (k_{obs}) for *p*-nitrophenyl acetate on the concentration of trifluoroethanol (T = 30°; Solv. H_2O; μ = 1.0 with KCl).

of hydroxide ion (Fig. 2). Thus, the reaction of trifluoroethanol with *p*-nitrophenyl acetate obeys the rate equation (30°, solvent H_2O, μ = 1.0 M with KCl)

$$\frac{-d(p\text{-NPA})}{dt} = \frac{k_T Ka}{K_W}(CH_3CH_2OH)(p\text{-NPA})(OH^-) = k_T(CF_3CH_2O^\ominus)(p\text{-NPA}) \quad (5)$$

where $k_T = 4.70 \pm 0.22 \times 10^4$ liter mole^{-1} min.$^{-1}$. The value of k_T for $CF_3CH_2O^\ominus$ compares, within an order of magnitude, to the previously determined k_T values for the reaction of *p*-nitrophenyl acetate with the oxide anions of *N*-acetylserinamide (Anderson *et al.*, 1961), pentaerythritol, and tris-(hydroxymethyl)-aminomethane (Bruice and York, 1961). We may conclude, therefore, that an internal acid catalysis (equation 4) is not essential to explain the magnitude of the transesterification rate constants for the reaction of alkoxide ions with *p*-nitrophenyl acetate.

The question remains whether log k_T is a linear function of the pKa′ of the alcohol involved. In Table II are recorded the second-order rate constants for the reaction of various oxide ion bases with *p*-nitrophenyl acetate and the pKa′ values for the corresponding conjugate acids. In Figure 3

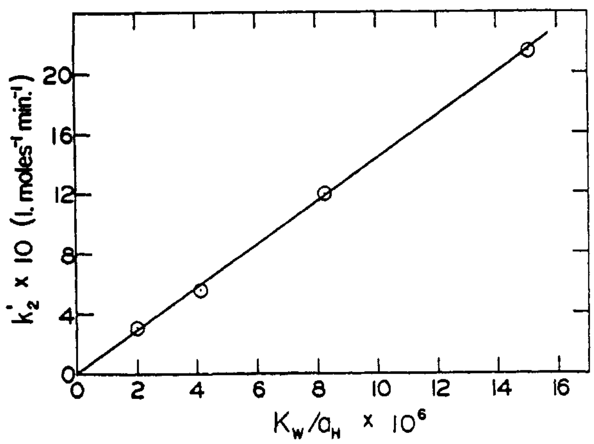


FIG. 2.—Linear dependence of *pseudo*-second-order velocity constant for the reaction of *p*-nitrophenyl acetate with CF_3CH_2OH on the concentration of hydroxide ion.

a Brönsted plot of pKa′ *vs.* log k_T is presented. Figure 3 includes $CH_3COO^\ominus$, *p*-$CHOC_6H_4O^\ominus$, $C_6H_5O^\ominus$, *p*-$ClC_6H_4O^\ominus$, $CF_3CH_2O^\ominus$, H_2NCO-CH-($NHCOCH_3$)$CH_2O^\ominus$ (shaded circle), and $(CH_2OH)_3$-$CCH_2O^\ominus$. The equation for the line correlating the pKa and log k_T values (average of over 10^{10} in Ka′) is

$$\log k_T = 0.76\,\mathrm{pKa} - 6.3 \tag{6}$$

and is essentially identical to that reported some years ago for the acetate and phenolate bases (Bruice and Lapinski, 1958).

By combining the rate equations for the reaction of alkoxide ion and $OH^\ominus$ with *p*-nitrophenyl acetate

$$v = \frac{k_T K_a}{H^+}(\mathrm{ROH})(p\text{-NPA}) \tag{7}$$

$$v = \frac{k_{OH} K_w}{H^+}(p\text{-NPA}) \tag{8}$$

with equation (6) we may obtain an expression for the concentration of total alcohol necessary for the rate of disappearance of ester via $OH^\ominus$ catalyzed hydrolysis and $RO^\ominus$ transesterification to be identical to

$$C_{ROH} = \frac{k_{OH} K_w}{5.5 \times 10^{-7}(K_a)^{0.24}} \tag{9}$$

Substituting the value of k_{OH} for *p*-nitrophenyl acetate in water at 30° (Bruice and Mayahi, 1960) and assuming the autoprotolysis constant of H_2O to be 10^{-14} provides

$$C_{ROH} = \frac{2.6 \times 10^{-5}}{(K_a)^{0.24}} \tag{10}$$

From (10) it is obvious that k_T for the reaction of any alkoxide ion with *p*-nitrophenyl acetate should be a readily measurable constant in nonbuffered solutions when $C_{ROH} = 1.0$ M. That this does not appear to be the case for alcohols of pKa′ greater than pK_w may be ascribed to the negative kinetic solvent effect encountered in aqueous solutions of normal alcohols (Potts and Amis, 1949; Amis and Jaffe, 1942; Bender and Glasson, 1959), to the difficulty in measuring pH in alcohol-water mixtures (Bates, 1954), or to operational difficulties due to the alteration of K_w with solvent composition (Bender and Glasson, 1959).

Also included in Table II are the second-order rate constants for the hydroxide ion-catalyzed hydrolysis (k_{OH}) of the acetyl esters of a series of alcohols of varying pKa′. In Figure 4 there is plotted log k_{OH} against the pKa′ of the various alcohols. Again it is seen that the rate of hydrolysis of *N,O*-diacetylserinamide (shaded circle) is in accord with the determined pKa′ of *N*-acetylserinamide. Just as there is nothing unusual about the nucleophilicity of the oxide anion of *N*-acetylserinamide there is also nothing unusual about the hydrolytic lability of the *O*-acyl bond of *N,O*-diacetylserinamide.

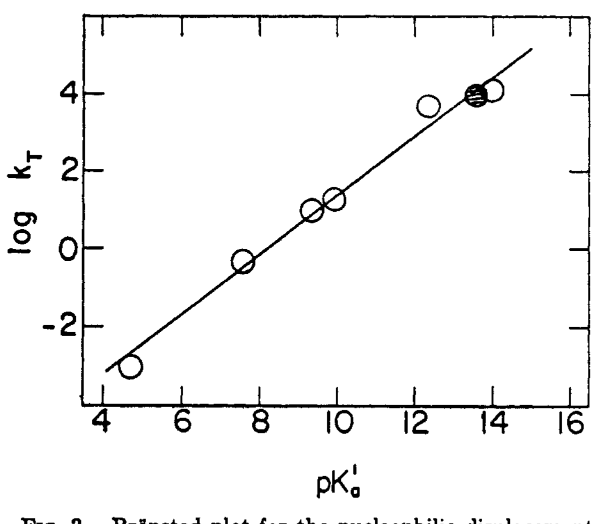


FIG. 3.—Brönsted plot for the nucleophilic displacement of *p*-nitrophenol from *p*-nitrophenyl acetate by various oxide avian bases. Shaded circle is point for *N*-acetylserinamide anion.

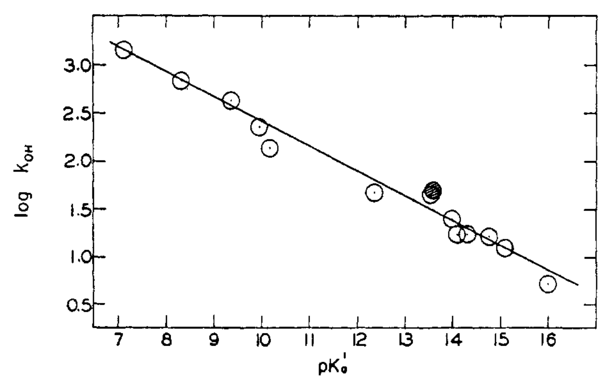


FIG. 4.—Linear dependence of the logarithm of the second-order rate constants for the alkaline hydrolysis of acetyl esters on the pKa′ of the corresponding alcohols. Shaded circle is for *N,O*-diacetylserinamide.

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Transpeptidation and the α-Chymotrypsin–Catalyzed Hydrolysis of α-Amino Acid Esters, Hydroxamides, Amides, and Hydrazides*

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Transpeptidation reactions have been shown to be of negligible, or minor, importance in the α-chymotrypsin–catalyzed hydrolysis of L-tyrosine methyl ester, hydroxamide, amide, and hydrazide, under conditions normally employed in kinetic studies. The kinetics of the hydrolytic reactions and their dependence upon pH can be interpreted in terms of participation of two substrate species, a less reactive α-amino acid derivative and its more reactive conjugate acid.

The apparent pH optima for the α-chymotrypsin–catalyzed hydrolysis of α-amino acid esters, hydroxamides, and hydrazides lie in a more acid region than those of the corresponding α-*N*-acyl derivatives. Results obtained with α-*N*-acylated and nonacylated derivatives of L-tyrosine (Kaufman *et al.*, 1949; Jansen *et al.*, 1951; Parks and Plaut, 1953; Schwert and Takenaka, 1955; Lutwack *et al.*, 1957) are summarized in Table I.

TABLE I
pH OPTIMA FOR α-CHYMOTRYPSIN–CATALYZED HYDROLYSES OF SOME L-TYROSINE DERIVATIVES

$-CH(CH_2C_6H_4OH)CO-$	$-OC_2H_5$	$-NH_2$	$-NHNH_2$	$-NHOH$
H_2N-	6.2–7.0[a,b]		7.05[f]	6.95[f]
CH_3CONH-	8.0–8.2[a,c]	7.90[f]	7.95[f]	7.60[f]
C_6H_5CONH-	7.8[d,e]	7.8[d,e]	8.0[f]	
β-$(C_5H_4N)CONH-$		7.90[f]	7.80[f]	

[a] Jansen *et al.* (1951). [b] Schwert and Takenaka (1955). [c] Parks and Plaut (1953). [d] Kaufman *et al.* (1949). [e] In 30% methanol. [f] Lutwack *et al.* (1957).

Foster *et al.* (1954) offered an explanation for the difference in pH optima of L-tyrosinhydroxamide and α-*N*-acetyl-L-tyrosinhydroxamide based upon the assumption that hydrolysis at the α-amino acid carboxyl function is the predominating reaction. Since the pH optima for uncharged substrates occur at pH 7.9 ± 0.1, it can be assumed that the enzyme is protonated at this pH in a manner that is optimal for catalyzing the hydrolysis of these substrates. The lower pH optimum of α-*N*-acetyl-L-tyrosinhydroxamide can be accounted for in terms of partial ionization of the latter substrate to the less reactive hydroxamate anion (Hogness and Niemann, 1953). However, the amino group of L-tyrosinhydroxamide, conjugate acid $pK_A' \cong 7.0$, would be predominately unprotonated at pH values much greater than 7. With an increase in concentration of the unprotonated species, assumed to be less reactive than the protonated or α-*N*-acylated-L-tyrosinhydroxamide, as the pH is increased, the pH optima would be shifted to a more acidic region than observed for the α-*N*-acyl derivative. This argument is equally applicable to the ester, amide, and hydrazide.

Schwert (1955) suggested that "it seems more probable that the reduction in apparent reaction velocity at higher pH values is attributable to transpeptidation onto the uncharged α-amino group." Support for this view is provided by observations that show that the extent of transpeptidation increases with increasing pH throughout the region of interest, *i.e.*, pH 6 to 8 (Johnston *et al.*, 1950a,b; Brenner *et al.*, 1950; Lestrovaya and Mardashev, 1956). Thus, there are three reactions to consider:

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