zation rates for cyclic and cycloalkyl phenyl ketones found in the present study are not a reflection of steric hindrance involving the base.

Application of the concepts involved in the stabilities of *exo* and *endo* double bonds in cyclic systems[19] and the factors involved in the ability of a carbocyclic ring to have one of its members undergo a coördination number change of four to three fail to correlate the present data.

One possible explanation that seems to agree with the experimental results centers around the position of the transition state along the reaction coördinate, and is concerned with the effect of hybridization of the ring carbons on the acidity of the hydrogens attached.

Cardwell and Kilner[7c] suggest that much more unsaturation develops, through partial carbonium ion formation, in the transition state for acid-catalyzed enolization than in the base-catalyzed reaction. It is therefore suggested that the transition state for base-catalyzed enolization occurs *early* in the process, and has its energy determined to a large extent by factors affecting the ketone, unlike that for acid-catalyzed enolization, which occurs much later and has its energy determined to a larger extent by steric and hybridization factors in the enol.

Coulson and Moffitt[34] have calculated the hybridization coefficients for a series of cycloalkanes, acetylene and ethane, with the results: cyclo-C_3H_6, 1.51; cyclo-C_4H_8, 1.67; cyclo-C_5H_{10}, 1.73; C_2H_6, 1.73; C_2H_4, 1.41; C_2H_2, 1.36. This coefficient reflects the s-character of the carbon orbital directed toward hydrogen and thus in part expresses the ionic character of the C–H bond.

This effect is, of course, evident in the carbonyl stretching frequencies for cycloalkanones which increase as one goes from cyclohexanone to cyclobutanone.[35] The effect is also reflected in the basicities of these ketones as measured by hydrogen bonding[36a] or by ultraviolet spectral shifts,[36b] $7 > 6 > 5 > 4$.

If one assumes a transition state that resembles factors (ketones) more than the product (enol), or one in which the α-carbon atom is predominantly sp^3-hybridized, one would conclude, combining Swain's postulated mechanism for enolization which involves long distances, and Pauli repulsion at the α-C–H unit, and the relative acidities of hydrogens attached to succeedingly smaller carbocyclic rings, that a sequence of exchange rates $(3) > 4 > 5 > 6 > (7)$ is consistent.

It is interesting that the cycloalkanones exchange more rapidly than the cycloalkyl phenyl ketones.

It is to be noted that in the changes in E_a and $\Delta S^{\ddagger}$, a facet other than just "acidity" of the α-hydrogen is to be detected. Presumably the fluctuations are to be attributed to solvation and steric effects. However, since strict compartmentalizing of solvent and steric effects into the energy parameter does not occur, it is at present not profitable to dissect the data further.

The reported equivalent acidity of cyclopropyl phenyl sulfone[37] and isopropyl phenyl sulfone is not relevant here since the comparison was made on an equilibrium (thermodynamic) basis rather than a kinetic one. Cyclopentyl phenyl sulfone and cyclohexyl phenyl sulfone exchange their α-H's for D at more nearly equal rates[38]; $k_5/k_6 \cong 1.5$. In the analogous ketones $k_5/k_6 = 6$.

Work in the sulfone area, which is currently in process, we hope will shed some light on the differences in α-H activation by carbonyl and sulfone groupings. The recent work by Cram,[39] indicating the markedly different steric courses followed by these substrates in D/H exchange reactions, suggests a distinct difference.

Acknowledgments.—R.E.D. wishes to thank Professors H. H. Jaffé and H. Zimmerman for several valuable discussions.

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[CONTRIBUTION NO. 165 FROM THE GRADUATE DEPARTMENT OF BIOCHEMISTRY, BRANDEIS UNIVERSITY, WALTHAM 54, MASS.]

The Nucleophilic Reactivity of Alcoholate Anions Toward *p*-Nitrophenyl Acetate

BY WILLIAM P. JENCKS AND MARY GILCHRIST

RECEIVED MARCH 8, 1962

The nucleophilic reactivity of a series of alcoholate anions toward the ester, *p*-nitrophenyl acetate, has been determined by measurement of reaction rates in dilute, buffered solutions or by measurement of product ratios after base-catalyzed solvolysis of the ester in alcohol–water mixtures. Strongly basic oxygen anions show only a slight increase in reactivity with increasing basicity, and thus deviate from the Brønsted relationship of slope 0.76–0.80 observed with less basic oxygen anions.

It was shown recently that, near neutral *p*H, the base-catalyzed reaction of *p*-nitrophenyl acetate with the hydroxyl group of N-acetylserinamide is much faster than the base-catalyzed hydrolysis or ethanolysis of this ester.[1] Bruice, *et al.*, found that the pK_a of N-acetylserinamide is 13.6 and that the reactivity of its anion toward *p*-nitrophenyl acetate does not deviate signifi-

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[1] B. M. Anderson, E. H. Cordes and W. P. Jencks, *J. Biol. Chem.* **236**, 455 (1961).

cantly from a "normal" Brønsted-type relationship of slope 0.76 obtained for other oxygen anions, including trifluoroethanol.[2] While the conclusion[2] that the hydrolysis is abnormally slow, rather than that the N-acetylserinamide reaction is abnormally fast, is probably reasonable, the high reactivity of N-acetylserinamide compared to ethanol is not accounted for by such a Brønsted relationship. Bruice, *et al.*, attributed this difference to a solvent effect, which might interfere with the measurement of the reaction with ethanol,[2] but it seemed unlikely that a solvent effect could account for a rate difference of this magnitude. The experiments reported here were initiated in an attempt to find the reason for this apparent discrepancy. The results indicate that strongly basic oxygen anions do not follow the Brønsted-type relationship found with weaker bases,[2-4] and that the nucleophilic reactivity of such strong bases is surprisingly insensitive to their basicity. A consequence of this insensitivity is that, at neutral or moderately basic pH, the base-catalyzed reactivity of acidic alcohols, including trifluoroethanol and N-acetylserinamide, is considerably greater than that of weakly acidic alcohols.

Experimental

Alcohols were redistilled after shaking with anhydrous Na_2CO_3 and standing over $CaCl_2$ or $CaSO_4$. Rate measurements were carried out as previously described.[4] Solutions of the alcohols in water were made up shortly before beginning the reaction and the reactions were complete within a few minutes, before appreciable decomposition of the alcohols occurred. Product ratios were determined after solvolysis of $1.3–2.0 \times 10^{-3}$ M p-nitrophenyl acetate in alcohol–water solutions maintained at an apparent pH of 10.6–11.0 with the aid of an automatic titrator (Radiometer TTT1c-SBR2c). The initial and final concentrations of ester were determined by conversion to hydroxamic acid[5] and the amount of acetate formed by hydrolysis was taken as the difference between the initial and final concentrations. There is approximately a 100-fold difference between the rates of alkaline hydrolysis of p-nitrophenyl acetate and of the esters formed as products, so that measurements could be made after complete solvolysis of p-nitrophenyl acetate and before appreciable hydrolysis of the product had occurred. Care was taken to avoid loss of volatile esters from the reaction mixture. The reactions of p-nitrophenyl acetate with water,[4] N-acetylserinamide[1] and trifluoroethanol[2] occur with only the anionic species of each reactant under conditions similar to those employed in these experiments; the ratio[4] k_{OH^-}/k_{H_2O} is 10^9. From these data and a number of unreported experiments at different pH values it was concluded that the neutral solvolysis of p-nitrophenyl acetate could be neglected under the conditions of these experiments.

Results

The rates of reaction of the more reactive alcohols with p-nitrophenyl acetate were measured directly in the presence of 0.2 M triethylamine buffer. The rate of the reaction with trifluoroethanol was shown to be unchanged in 0.1 M buffer. The observed rates were found to be linear in respect to alcohol concentration (Fig. 1), indicating the absence of large solvent effects on the rate in the dilute alcohol solutions used for these experiments. The specific rate constants for the alcoholate anions were calculated from the observed rates and the known acidities of the alcohols (Table I). No activity coefficient corrections were made, but this would not appear to introduce a large error with the uncharged alcohols, since similar rate constants were obtained from experiments in buffer alone and in solutions brought to an ionic strength of 1.0 with KCl. A larger effect of ionic strength

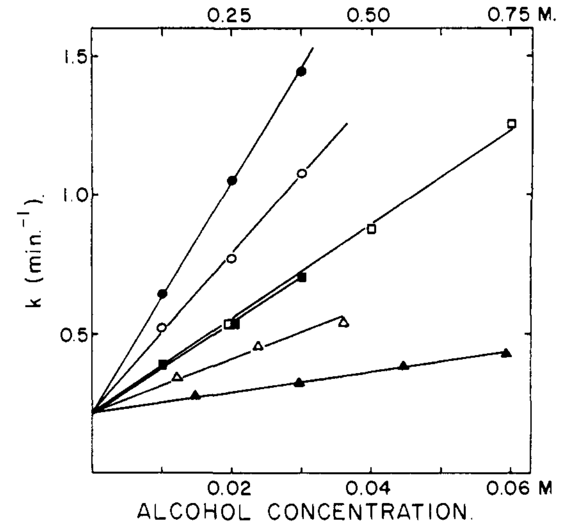

Fig. 1.—The reaction of p-nitrophenyl acetate with alcohols in 0.2 M triethylamine buffer, pH 10.41 ± 0.02 at 25°: lower scale: ●, trifluoroethanol; ○, trichloroethanol; □, 2-propynol; ■, 2,2,3,3-tetrafluoropropan-1-ol; upper scale: △, 2-chloroethanol; ▲, methanol.

TABLE I

RATE CONSTANTS FOR THE REACTIONS OF ALCOHOLATE ANIONS WITH p-NITROPHENYL ACETATE AT 25°

Alcohol	pK_a'[a]	Concn. range, M	k_2,[b] M^{-1} min.$^{-1}$	k_2,[b,c] M^{-1} min.$^{-1}$
From rate measurements				
Phenol[d]	10.0	0.05–0.20	67	58
2,2,2-Trichloroethanol	12.24	.01– .03	2,000	1,850
2,2,2-Trifluoroethanol	12.37	.01– .03	3,850	3,600
2,2,3,3-Tetrafluoropropan-1-ol	12.74	.01– .03	3,630	3,640
2-Propyn-1-ol	13.55	.02– .06	25,000	21,500
Choline	13.9	.025–.10	12,300	8,000
2-Chloroethanol	14.31	.15– .45	6,500	5,600
Methanol	15.5	.19– .74	36,000	29,000
Hydroxide ion[e]	15.75		890	
From product ratios				
			k_2[f]	
2-Methoxyethanol	14.8	0.64–1.59	5,300	
Methanol	15.5	0.33–1.86	41,000	
Ethanol	16.0	1.74–4.35	15,200	

[a] References 6 and 7. [b] Determined in 0.20 M triethylamine buffer, 25% free base. The experimental rate constants are reproducible to less than ±10%; activity coefficients and dissociation constants introduce a greater uncertainty into the calculated values of k_2 (see text). [c] Ionic strength maintained at 1.0 with KCl. [d] No triethylamine added; determined in phenol buffers, 10% phenolate anion. [e] At ionic strength 0.3; included for comparison.[4] [f] Determined at an apparent pH of 10.6.

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(2) T. C. Bruice, T. H. Fife, J. J. Bruno and N. E. Brandon, *Biochemistry*, **1**, 7 (1962).

(3) T. C. Bruice and R. Lapinski, *J. Am. Chem. Soc.*, **80**, 2265 (1958).

(4) W. P. Jencks and J. Carriuolo, *ibid.*, **82**, 1778 (1960).

(5) S. Hestrin, *J. Biol. Chem.*, **180**, 249 (1949).

(6) P. Ballinger and F. A. Long, *J. Am. Chem. Soc.*, **82**, 795 (1960).

(7) R. M. C. Dawson, D. C. Elliott, W. H. Elliott and K. M. Jones, "Data for Biochemical Research," Oxford Univ. Press, London, 1959, p. 8.

was observed with the charged alcohol, choline, and the rate constants for this compound, although included in Table I, should not be regarded as more than approximations. The rate constants for phenolate were redetermined in phenol–phenolate buffers, since previous, divergent values[3,4] were based on pK_a values determined under conditions different from those of the rate measurements. No reaction was observed in triethylamine buffer with up to 0.7 M formamide or 0.3 M sodium potassium tartrate. The rate constant of 3850 M^{-1} min.$^{-1}$ obtained for trifluoroethanol at 25° may be compared with the reported[2] value of 4700 M^{-1} min.$^{-1}$ at 30°.

The rates of reaction of less reactive alcohols, such as ethanol, cannot be measured in this manner since no rate increase is observed with dilute solutions of alcohol in water[1] and solvent effects may complicate the interpretation of rate experiments in more concentrated solutions.[2] The relative reactivities of alcohol and water in base-catalyzed reactions toward p-nitrophenyl acetate were, therefore, determined by measuring the amounts of acetate and ethyl acetate formed after solvolysis of the ester in alkaline alcohol–water mixtures. Alcohol was present in large excess compared to the concentration of ester, so that the alcohol concentration was essentially constant during the reaction and the disappearance of ester followed pseudo-first-order kinetics. If it is assumed that solvent effects on the concentration and reactivity ratios of RO^- and HO^- are small, then the rate constant for reaction of a dilute solution of alcoholate anion in water under those conditions may be estimated from eq. 1, derived from the rate and equilibrium constants for the individual reactions

$$k_{\mathrm{RO}^-} = \frac{k_{\mathrm{OH}^-}K_{\mathrm{W}}}{55.5K_{\mathrm{ROH}}}\frac{[\mathrm{H_2O}]}{[\mathrm{ROH}]}R \qquad (1)$$

in which k_{RO^-} and k_{OH^-} are the rate constants for the reactions with alcoholate and hydroxide anions, K_{W} is the autoprotolysis constant for water, K_{ROH} is the dissociation constant for the alcohol and R is the observed ratio of alcoholysis to hydrolysis, from the ratio of products. The value of k_{OH^-} is taken[4] as 890 M^{-1} min.$^{-1}$ at 25° and K_{W} as 10^{-14}. The results of a typical experiment with methanol are given in Table II. It is not unreasonable to assume that solvent effects on these concentration and reactivity *ratios* are small at the alcohol concentrations used in these experiments, and this assumption is experimentally supported by (1) the reasonable agreement between the rate constants obtained by direct measurement and by measurement of product ratios for the reaction with methoxide ion (Table I); (2) the absence of a trend in the calculated rate constants with increasing alcohol concentration for the methanol reaction (Table II) and for the other alcohols examined; and (3) the insensitivity to solvent composition, at considerably higher methanol and ethanol concentrations, of the corresponding ratios of rate constants for the reactions of methoxide, ethoxide and hydroxide with acetyl-L-phenylalanine methyl and ethyl esters[8]; and (4) the insensitivity of the order and approximate ratio of acid strengths of structurally similar compounds to variation in solvent.[6,9] While the rate constants obtained by this method are not exact, they are of sufficient accuracy for the purposes of this work and it is likely that the uncertainty of the dissociation constants is larger than that introduced by these assumptions.

TABLE II

THE REACTION OF METHANOL WITH p-NITROPHENYL ACETATE AT 25.0°. ESTIMATION OF RATES FROM PRODUCT RATIOS

Methanol, M	$\frac{\text{Acetate}}{\text{Methyl acetate}}$	A^a	k_{MeO^-},[b] M^{-1} min.$^{-}$
0.33	2.0	83	42,000
.50	1.32	83	42,000
.74	0.92	79	40,000
1.24	.51	83	42,000
1.86	.35	79	40,000

[a] A = $[\mathrm{H_2O}]$ [methyl acetate]/[EtOH] [acetate]; p-nitrophenyl acetate = 1.33×10^{-3} M; apparent pH 10.6.
[b] See text for method of calculation.

The higher reactivity of ethoxide than of hydroxide toward p-nitrophenyl acetate prompted a re-examination of the relative reactivities toward acetylimidazole.[1] The previously reported values were found to be low, due to losses of ethyl acetate, and the ratio of reactivities of ethanol and water in base-catalyzed reactions with acetylimidazole was found to be approximately 40. This does not alter the previous conclusions[1] that, near neutral pH, the base-catalyzed reaction of N-acetylserinamide with acetylimidazole is much faster than that of ethanol and that the reaction of ethanol with acetylimidazole is subject to general base catalysis by imidazole.

Discussion

The reactivity of strongly basic oxygen anions toward p-nitrophenyl acetate is remarkably insensitive to the basicity of the anion. The anions of trifluoroethanol, tetrafluoropropanol, N-acetylserinamide,[1,2] chloroethanol and methoxyethanol vary 250-fold in basicity, but differ by less than twofold in reactivity. The anions of 2-propynol and methanol are more reactive because of their decreased steric hindrance, but the reactivities of these two compounds differ from each other by less than twofold in spite of a 100-fold difference in basicity. All of the compounds with a pK_a greater than 12 are more reactive than hydroxide ion and the rate constants for the reactions of ethoxide and methoxide ions are 170 to 450 times greater than that for hydroxide ion. It is of interest that the relative reactivities of hydroxide, ethoxide and methoxide ions toward acetyl-L-phenylalanine methyl and ethyl esters, compounds with a poorer leaving group than p-nitrophenyl acetate, vary over a range of only approximately fourfold.[8]

A logarithmic plot of reactivity against basicity is shown in Fig. 2. An arbitrary line of slope 0.76 is drawn through the point for phenolate, since the reactivity of less basic oxygen anions, including phenolate, approximately follows such a line.[2-4] The insensitivity of the reactivity of basic oxygen

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(8) M. L. Bender and W. A. Glasson, *J. Am. Chem. Soc.*, **81**, 1590 (1959).

(9) R. P. Bell, "The Proton in Chemistry," Cornell University Press, Ithaca, N. Y., 1959, p. 58.

anions to basicity is reflected in the negative deviations of such anions from this line. It would appear that the well-known low reactivity of hydroxide ion is also exhibited, although to a lesser extent, by other strongly basic oxygen anions.

If the reactivity of a series of bases is measured at a *p*H below the pK_a of their conjugate acids, it is commonly found that there is only a relatively small decrease in the observed reaction rates with increasing basicity of the nucleophilic reagent. This is because the smaller concentration of the more basic reagent present at a given *p*H value is largely compensated for by its increased reactivity. In the present case, the low concentrations of the anions of weakly acidic alcohols is not compensated for by an increased reactivity and, as a result, the base-catalyzed reactions of such alcohols in neutral or moderately basic solution are slow compared with those of more acidic alcohols. This provides an explanation for the apparent very high reactivity of acidic alcohols, such as trifluoroethanol and N-acetylserinamide, relative to ethanol or to base-catalyzed hydrolysis.

The two requirements for effectiveness of a nucleophilic catalyst are (i) that it have a higher reactivity toward the substrate than the ultimate acyl acceptor and (ii) that the intermediate acylated product be highly reactive toward the final acyl acceptor. The reactivity of the anion of N-acetylserinamide and, presumably, the intrinsic reactivity of the anion of the serine hydroxyl group of chymotrypsin and related enzymes is higher than that of hydroxide ion, in spite of the thousand-fold greater basicity of hydroxide ion. In this respect, then, the serine hydroxyl group, by virtue of its relatively high acidity, is a good candidate for the active site of an enzyme which carries out nucleophilic catalysis. It is not better in this respect than imidazole or a phenolate ion, but it has the advantage that the product of nucleophilic reaction is presumably a relatively "low energy" compound so that, in contrast to the corresponding products from imidazole and phenol, there is not a large thermodynamic barrier to its formation. Bruice, *et al.*,[2] have confirmed the previous finding[1] that N,O-diacetylserinamide, the product of the nucleophilic reaction, is not unusually reactive toward hydroxide ion. It is, however, at least several orders of magnitude more reactive than ethyl acetate toward nitrogen-containing nucleophilic reagents in base-catalyzed reactions, and this may contribute to the rapid rate of enzyme-catalyzed acyl transfer reactions to nitrogen-containing acyl-acceptors.[1]

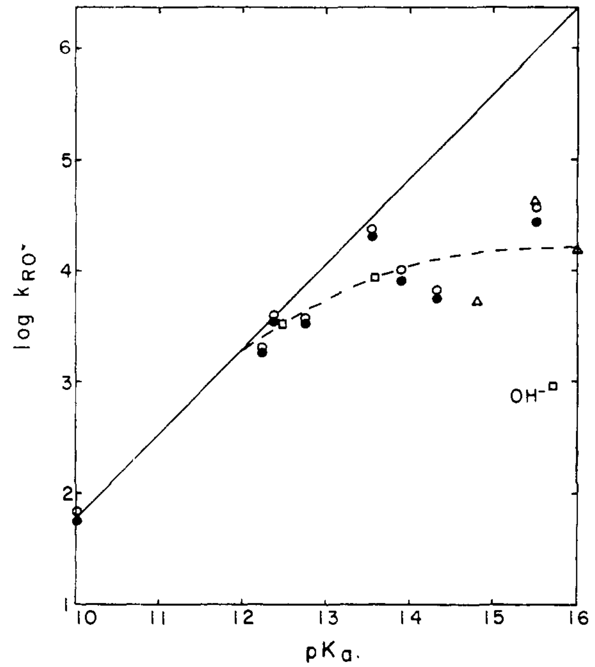

Fig. 2.—Reactivity of alcoholate anions toward *p*-nitrophenyl acetate at 25.0° as a function of pK_a': ○, 0.2 *M* triethylamine buffer; ●, 0.2 *M* triethylamine buffer maintained at ionic strength 1.0 with KCl (no triethylamine used in phenol experiments); △, from product ratios; □, previously reported data[1,2,4] for acetoxime anion, N-acetylserinamide anion and hydroxide ion. The other compounds shown are listed in Table I.

We do not have a satisfactory mechanistic explanation for the low sensitivity to basicity of the reactivity of strongly basic oxygen anions. Solvation of these ions is undoubtedly of great importance to both their reactivity and basicity, but sufficient data to evaluate the effects of solvation on these properties are not available. Their low reactivity might be regarded as an extreme expression of the Hammond postulate[10]; *i.e.*, for highly reactive compounds the transition state may be reached early in the course of the reaction and intrinsic bond-forming ability may have less opportunity for expression. It is doubtful, however, that this effect can account for the magnitude of the observed results.

Acknowledgments.—We are grateful to Dr. Thomas Bruice for informing us of his results before publication and to the National Cancer Institute of the National Institutes of Health (Grant C-3975) and the National Science Foundation for financial support.

(10) G. S. Hammond, *J. Am. Chem. Soc.*, **77**, 334 (1955).