Nucleophilic Catalysis by Acetate Ion in the Methanolysis of *p*-Nitrophenyl Acetate[1,2]

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Abstract: *p*-Nitrophenyl acetate undergoes methanolysis, catalyzed by tritium-labeled acetate ion, to yield *p*-nitrophenol and methyl acetate, which exhibits a specific radioactivity 0.56 ± 0.06 that of the acetate catalyst. The reaction therefore proceeds solely by way of an acetic anhydride intermediate (nucleophilic catalysis) in methanol, whereas the hydrolysis of the same substrate in water has been shown by Oakenfull, Riley, and Gold to occur with 56% nucleophilic and 44% protolytic catalysis. The nucleophilic reaction in methanol shows $\Delta H^* = 11 \pm 1$ kcal/mol and $\Delta S^* = -39 \pm 3$ eu.

The hydrolysis of reactive carboxylic acid esters (eq 1, R = H) is accelerated by carboxylate ions.[4] Schemes I and II are two ways of accounting for this

$$CH_3CO_2Ar + ROH \longrightarrow CH_3CO_2R + ArOH \quad (1)$$

Scheme I

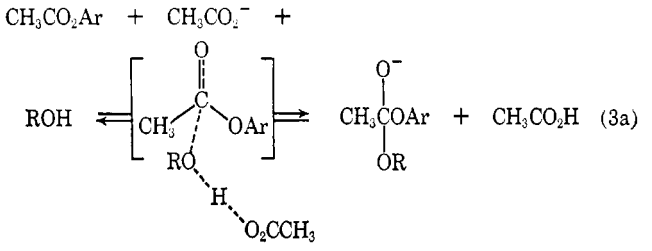
$$CH_3CO_2Ar + CH_3CO_2^- \rightleftharpoons CH_3\overset{\overset{O^-}{|}}{C}OCOCH_3 \longrightarrow$$
$$\underset{\mathbf{1}}{\overset{|}{OAr}}$$

$$CH_3CO_2COCH_3 + ArO^- \quad (2a)$$

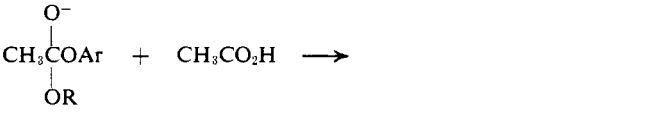
$$CH_3CO_2COCH_3 + ROH \longrightarrow CH_3CO_2R + CH_3CO_2H \quad (2b)$$

$$CH_3CO_2H + ArO^- \longrightarrow CH_3CO_2^- + ArOH \quad (2c)$$

Scheme II



$$CH_3CO_2Ar + CH_3CO_2^- + ROH \rightleftharpoons \text{[tetrahedral intermediate]} \rightleftharpoons CH_3\overset{\overset{O^-}{|}}{C}OAr + CH_3CO_2H \quad (3a)$$
$$\underset{OR}{|}$$



$$CH_3\overset{\overset{O^-}{|}}{C}OAr + CH_3CO_2H \longrightarrow CH_3CO_2R + ArOH + CH_3CO_2^- \quad (3b)$$
$$\underset{OR}{|}$$

observation. In Scheme I (nucleophilic catalysis), the acetate ion functions by conversion of the substrate into the more reactive substance, acetic anhydride, which then is rapidly transformed to products with regeneration of the catalyst. Scheme II (protolytic catalysis, general base catalysis, or classical general base catalysis) represents removal by the catalyst of a proton from an attacking solvent molecule during generation of the tetrahedral intermediate. Other versions of this mechanism would show different steps as rate determining but these are not distinguishable from Scheme II by methods to be discussed in this paper.

Oakenfull, Riley, and Gold[5] recently published a determination of the contributions made by each of these types of catalysis to the acetate-catalyzed hydrolyses of various aryl acetates in aqueous solution. Nucleophilic catalysis was measured by trapping the acetic anhydride intermediate with aniline or *m*-toluidine. For *p*-nitrophenyl acetate, nucleophilic catalysis and protolytic catalysis were nearly equally balanced, the former accounting for 56–58% of the effect of the acetate ion.

The use of some general-catalyzed ester cleavage reactions as models for esteratic enzyme action[6] lends some importance to a study of the general-catalyzed alcoholysis of esters, because these enzymes appear to cleave esters by acylation of a serine hydroxyl group of the active site (an alcoholysis reaction) and subsequent hydrolysis of the acyl enzyme.[7]

The methanolysis of picryl and 2,4-dinitrophenyl acetates is subject to general catalysis by pyridine, 2-picoline, 2,6-lutidine, and 2,4,6-collidine. The Brønsted relation is not obeyed but instead a retardation by 2-substituents is seen, which was taken as a steric effect indicative of a nucleophilic mode of catalysis by these bases.[8] On the other hand, the ethanolysis of ethyl trifluoroacetate appears to be subject to protolytic catalysis since 2,6-lutidine and pyridine show the same catalytic constant.[9] Such a change in catalytic mode to a protolytic mechanism with poor leaving groups is to be expected,[5] since the poorer the leaving group the greater the fraction of intermediate **1** (eq 2a) which will revert to reactants.[10] The way is thus opened for competition by Scheme II.

Much more readily than in the case of hydrolysis, a direct experimental dissection of the catalytic activity

(1) Catalysis in Ester Cleavage. I. For part II, see R. L. Schowen, C. G. Mitton, M. Gresser, and J. Shapley, to be published.

(2) This work was supported by a grant from the National Science Foundation (GP6397) and an allocation from the Biomedical Science Support Grant of the University of Kansas. Further details may be found in C. G. Behn, M.S. Thesis, University of Kansas, 1967.

(3) National Defense Education Act Fellow.

(4) The subject has been reviewed by (a) M. L. Bender, *Chem. Rev.*, **60**, 53 (1960); (b) S. L. Johnson, *Advan. Phys. Org. Chem.*, **5**, 237 (1967); (c) T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms," Vol. 1, W. A. Benjamin, Inc., New York, N. Y., 1966, p 27 ff.

(5) D. G. Oakenfull, T. Riley, and V. Gold, *Chem. Commun.*, 385 (1966).

(6) H. R. Mahler and E. H. Cordes, "Biological Chemistry," Harper and Row, New York, N. Y., 1966, p 297 ff. In this paper, "general catalysis" refers to acceleration by species in addition to lyate ion, by whatever mechanism.

(7) Reference 6, pp 290, 311–312.

(8) (a) W. R. Ali, A. Kirkien-Konasiewicz, and A. Maccoll, *Chem. Ind.* (London), 909 (1964); (b) A. Kirkien-Konasiewicz and A. Maccoll, *J. Chem. Soc.*, 1267 (1964); (c) W. R. Ali, A. Kirkien-Konasiewicz, and A. Maccoll, *ibid.*, 6409 (1965).

(9) S. L. Johnson, *J. Am. Chem. Soc.*, **86**, 3819 (1964).

(10) J. F. Kirsch and W. P. Jencks, *ibid.*, **86**, 833 (1964).

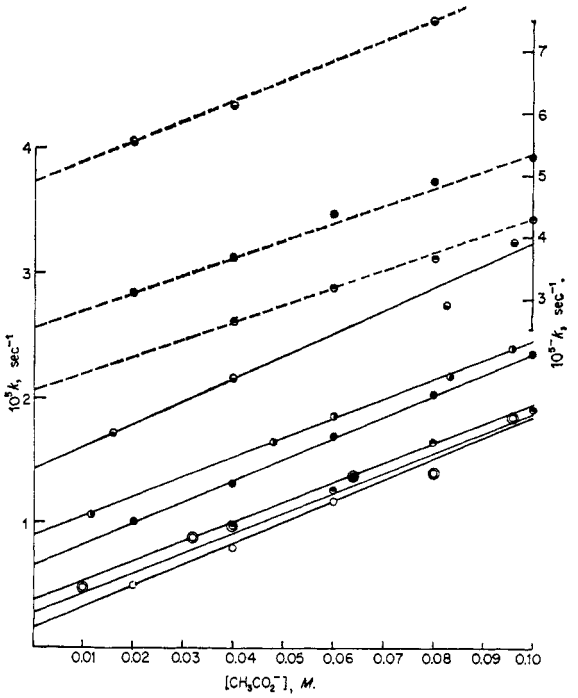


Figure 1. First-order rate constants for methanolysis of *p*-nitrophenyl acetate as a function of acetate ion concentration in sodium acetate–acetic acid buffers. The solid lines and their associated points refer to the reaction at 27.4 ± 0.1°; the dashed lines and associated points to that at 36.6 ± 0.1°. The left-hand ordinate is labeled for the data at 27.4°, and the right-hand ordinate for those at 36.6°. The buffer ratios represented are: $[CH_3CO_2^-]/[CH_3CO_2H]$ = 0.25 (○); 0.50 (◓); 0.67 (◎); 1.00 (●); 1.50 (◑); and 2.0 (◒). Reading up from the bottom of the figure, the lines fit points for the buffer ratios of 0.25, 0.67, 0.50, 1.00, 1.50, and 2.00 (all at 27.4°), and 0.50, 1.00, and 2.00 (all at 36.6°).

of carboxylate ions in alcoholysis into nucleophilic and protolytic components may be effected. Consider the methanolysis of *p*-nitrophenyl acetate, catalyzed by radioactive acetate ion. Equations 4 and 5 show the fate of the label[11] in Schemes I and II. If such an

$$CH_3CO_2Ar + CH_3^*CO_2^- \longrightarrow CH_3^*CO_2COCH_3 + ArO^- \quad (4a)$$

$$CH_3^*CO_2COCH_3 + CH_3OH \longrightarrow 0.5CH_3^*CO_2CH_3 + 0.5CH_3CO_2CH_3 + 0.5CH_3CO_2H + 0.5CH_3^*CO_2H \quad (4b)$$

$$0.5CH_3CO_2H + 0.5CH_3^*CO_2H + ArO^- \longrightarrow 0.5CH_3CO_2^- + 0.5CH_3^*CO_2^- + ArOH \quad (4c)$$

$$CH_3CO_2Ar + CH_3^*CO_2^- + CH_3OH \rightleftharpoons CH_3\overset{O^-}{\overset{|}{C}}OAr + CH_3^*CO_2H \quad (5a)$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad OCH_3$$

$$CH_3\overset{O^-}{\overset{|}{C}}OAr + CH_3^*CO_2H \longrightarrow CH_3CO_2CH_3 + ArOH + CH_3^*CO_2^- \quad (5b)$$
$$\qquad\quad OCH_3$$

(11) The labeled acetic anhydride is assumed to undergo methanolysis at both carbonyl groups at equal rates (neglecting a secondary isotope effect due to the tracer label), whence the coefficients of one-half in eq 4b. In the work to be reported below, the label was a single tritium atom. M. L. Bender and M. S. Feng (*J. Am. Chem. Soc.*, 93, 6318 (1960)) found that acetic anhydride underwent hydrolysis at pH 5.3 in aqueous solution at 20° 1.05 ± 0.04 times faster than acetic anhydride-d_6. Roughly speaking, this corresponds to a tritium isotope effect (k_H/k_T for one tritium atom) of about 1.01.

excess of catalyst over substrate is used that no sensible dilution of the label is caused by nucleophilic catalysis, then purely nucleophilic catalysis will generate methyl acetate with one-half the specific activity of the catalyst, while purely protolytic catalysis would give rise to no activity at all in the methyl acetate product. Both mechanisms will of course exhibit kinetic orders of unity in both catalyst and substrate. If k_N is the over-all second-order rate constant for nucleophilic catalysis and k_P the over-all second-order rate constant for protolytic catalysis, then the catalytic constant for acetate ion, k_C, will then be the sum of the rate constants for the two component modes (eq 6). Now

$$k_C = k_N + k_P \quad (6)$$

radioactive methyl acetate may be formed in a single way only: by attack of methanol on labeled acetic anhydride (which can only be formed in a nucleophilic reaction of labeled acetate ion, C*) at the carbonyl group nearer the label. All other processes lead to unlabeled methyl acetate. The ratio of labeled methyl acetate (E*) to unlabeled methyl acetate (E) is equal to the ratio of the rates of their formation (eq 7). Tracer

$$\frac{[E^*]}{[E]} = \frac{0.5k_N[C^*]}{0.5k_N[C]^* + k_C[C] + k_P[C^*] + k_U} \quad (7a)$$

$$\frac{[E]^*}{[E]} = \frac{0.5k_N[C^*]}{k_C[C] + k_U} = \frac{0.5k_N[C^*]/[C]}{k_C + k_U/[C]} \quad (7b)$$

levels of label are assumed; the k_U term covers routes to methyl acetate not catalyzed by acetate ion. Since the fraction of labeled compound is proportional to the specific activity of the substance (eq 8),[12] eq 7 can be solved for k_N and k_P in terms of known quantities.

$$S_E = \alpha([E^*]/[E]) \quad (8a)$$

$$S_A = \alpha([C^*]/[C]) \quad (8b)$$

$$k_N = 2(S_E/S_A)\{k_C + k_U/[C]\} \quad (9a)$$

$$k_P = k_C - k_N \quad (9b)$$

We report here such a study of the acetate-catalyzed methanolysis of *p*-nitrophenyl acetate.

Results

Kinetics. Good first-order kinetics were observed for the conversion of *p*-nitrophenyl acetate (2) into methyl acetate and *p*-nitrophenol in lithium acetate–acetic acid buffers. Table I contains the observed rate constants as a function of buffer composition at 27.4 and 36.6°. The rate constants are linear in acetate ion concentration at constant pH, indicating general catalysis, as shown in Figure 1. The slopes of the lines in the figure are independent of the acetic acid concentration; the acetate ion thus functions as a general base while no catalysis by acetic acid is found. The intercepts of Figure 1 are linear in methoxide ion concentration (the plots are not shown). The observations may be summarized by the kinetic law of eq 10, for which values of the constants are given in Table II.

$$-d[S]/dt = [S]\{k_0 + k_B[CH_3O^-] + k_{Ac}[CH_3CO_2^-] + k_{HAc}[CH_3CO_2H]\} \quad (10)$$

(12) The proportionality constant α involves constants of the system and the instruments. Special precautions, described in the Experimental Section, were taken to ensure that α was equal for E and C.

Table I.[a] First-Order Rate Constants for the Methanolysis of *p*-Nitrophenyl Acetate in Acetic Acid–Lithium Acetate Buffers at 27.4 ± 0.1 and 36.6 ± 0.1°

Temp, °C	$[CH_3CO_2^-]$, *M*	$[CH_3CO_2H]$, *M*	$10^5[CH_3O^-]$, *M*[c]	10^5k,[b] sec^{-1}
27.4	0.060	0.240	0.83	1.19 ± 0.02
	0.040	0.160	0.83	0.80 ± 0.05
	0.020	0.080	0.83	0.51 ± 0.05
27.4	0.100	0.200	1.65	1.92 ± 0.01
	0.080	0.160	1.65	1.66 ± 0.02
	0.060	0.120	1.65	1.37 ± 0.01
	0.040	0.080	1.65	0.98 ± 0.01
27.4	0.096	0.144	2.20	1.86 ± 0.02
	0.080	0.120	2.20	1.40 ± 0.08
	0.064	0.096	2.20	1.39 ± 0.02
	0.040	0.060	2.20	0.98 ± 0.02
	0.032	0.048	2.20	0.79 ± 0.03
	0.016	0.024	2.20	0.49 ± 0.01
27.4	0.100	0.100	3.30	2.36 ± 0.04
	0.080	0.080	3.30	2.03 ± 0.03
	0.060	0.060	3.30	1.70 ± 0.01
	0.040	0.040	3.30	1.31 ± 0.05
	0.020	0.020	3.30	1.02 ± 0.02
27.4	0.096	0.064	4.95	2.40 ± 0.04
	0.084	0.056	4.95	2.18 ± 0.02
	0.060	0.040	4.95	1.87 ± 0.03
	0.048	0.032	4.95	1.65 ± 0.05
	0.012	0.008	4.95	1.07 ± 0.04
27.4	0.096	0.048	6.60	3.27 ± 0.09
	0.080	0.040	6.60	2.75 ± 0.09
	0.040	0.020	6.60	2.17 ± 0.09
	0.016	0.008	6.60	1.73 ± 0.07
36.6	0.100	0.200	*d*	4.32 ± 0.08
	0.080	0.160		3.68 ± 0.06
	0.060	0.120		3.20 ± 0.05
	0.040	0.080		2.65 ± 0.07
36.6	0.100	0.100		5.32 ± 0.10
	0.080	0.080		4.94 ± 0.07
	0.060	0.060		4.40 ± 0.09
	0.040	0.040		3.69 ± 0.05
	0.020	0.020		3.11 ± 0.04
36.6	0.080	0.040		7.51 ± 0.12
	0.040	0.020		6.14 ± 0.08
	0.020	0.010		5.57 ± 0.07

[a] Ionic strength maintained at 0.100 *M* by addition of lithium chloride or lithium perchlorate. [b] Error limits are standard deviations. [c] Calculated from pK_a (CH_3CO_2H) = 9.44 (ref 13) and pK_{auto} (CH_3OH) = 16.92 (E. Grunwald and E. Price, *J. Am. Chem. Soc.*, **86**, 4517 (1964)). [d] pK_{auto} (CH_3OH) is not known at 36.6°.

Table II. Catalytic Constants for the Methanolysis of *p*-Nitrophenyl Acetate in Acetic Acid–Lithium Acetate Buffers at 27.4 ± 0.1 and 36.6 ± 0.1° (μ = 0.100 *M*)

Constant	27.4°	36.6°
k_{HAc}	0 ± 0.1 × 10^{-4} M^{-1} sec^{-1}	0 ± 0.2 × 10^{-4} M^{-1} sec^{-1}
k_{Ac}	1.6 ± 0.1 × 10^{-4} M^{-1} sec^{-1}	3.0 ± 0.2 × 10^{-4} M^{-1} sec^{-1}
k_B	220 ± 100 M^{-1} sec^{-1}	*a*
k_0	−5 ± 7 × 10^{-6} sec^{-1}	3.9 ± 0.3 × 10^{-6} sec^{-1}

[a] Since the autoprotolysis constant of methanol at 36.6° ($K_{auto}^{36.6}$) is unknown, only $k_BK_{auto}^{36.6}$ = 2.23 ± 0.04 × 10^{-5} sec^{-1} was determined.

Tracer Experiments. According to the method outlined in the introduction, the incorporation of label from the acetate catalyst into the product methyl acetate was determined. Aliquots were removed from a reaction solution, quenched, and subjected to a series of microdistillations which effectively isolated the methyl acetate product for scintillation counting. Under the conditions used for counting, we can write for the observed count p_{obsd} (in counts per minute)

$$p_{obsd} = m_E S_E \tag{11}$$

where m_E is the number of moles of methyl acetate removed in the aliquot (and now present in the counting solution) and S_E is the specific activity of the methyl acetate, in terms of counts per minute per mole, again under the specified counting conditions. If v is the volume in liters of the aliquot, $[S]_0$ the initial molar concentration of *p*-nitrophenyl acetate, and k the first-order rate constant under the reaction conditions used (0.100 *M* acetate ion, 0.400 *M* acetic acid), then

$$m_E = [S]v = [S]_0(1 - e^{-kt})v \tag{12}$$

The desired specific activity of the methyl acetate, S_E, is thus given by eq 13. The data for calculation of S_E

$$S_E = \frac{p_{obsd}}{[S]_0(1 - e^{-kt})v} = \frac{p_{obsd}/[S]_0v}{\text{fraction of reaction}} \tag{13}$$

at points from 30 to 90% reaction are given in Table III. The resulting average value is S_E = 12.0 ± 0.7 × 10^{10} cpm/mol when the catalyst activity is S_A = 21.4 ± 0.5 × 10^{10} cpm/mol. The degree of incorporation is thus S_E/S_A = 0.56 ± 0.06.

Table III. Incorporation of Radioactivity into Methyl Acetate Product during the Course of the Methanolysis of *p*-Nitrophenyl Acetate Catalyzed by 0.10 *M* Tritium-Labeled Acetate Ion (S_A = 21.4 ± 0.5 × 10^{10} cpm/mol)

Fraction of reaction	$10^{-10}(p_{obsd}/[S]_0v)$, cpm/mol	$10^{-10}S_E$, cpm/mol
0.30	3.36	11.2
0.41	4.96	12.3
0.61	7.43	12.3
0.76	10.2	13.4
0.80	9.24	11.5
0.90	10.3	11.4

Under the conditions used, the uncatalyzed term (k_U in eq 9a) is 12 ± 7 × 10^{-7} sec^{-1}. Application of eq 9 yields k_N = 1.9 ± 0.3 × 10^{-4} M^{-1} sec^{-1} and k_P = −0.3 ± 0.4 × 10^{-4} M^{-1} sec^{-1}.

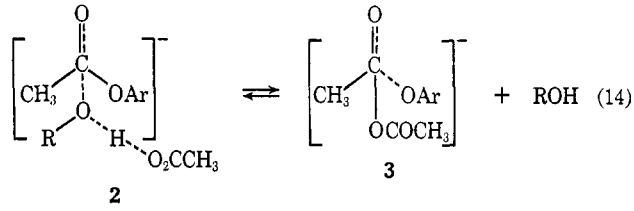
Activation Parameters. From the data of Table II, the enthalpy and entropy of activation for the acetate ion catalyzed methanolysis of *p*-nitrophenyl acetate are ΔH^* = 11 ± 1 kcal/mol and ΔS^* = −39 ± 3 eu for standard states of 1 *M* for solutes and pure liquid for methanol. If it is assumed that the reaction goes entirely by the nucleophilic route at 36.6° as well as at 27.4°, these are the activation parameters for nucleophilic catalysis.

Discussion

Mechanism of the Reaction. The extent of incorporation of radioactive label from catalyst acetate ion into product methyl acetate in the methanolysis of *p*-nitrophenyl acetate is consistent with entirely nucleophilic catalysis, with no detectable component of protolytic catalysis. Scheme I above therefore appears adequately to describe the system. It seems likely that acetate ion would depart from the tetrahedral intermediate **1** more rapidly than would *p*-nitrophenoxide since the latter is a stronger base (the pK_a of acetic acid is 9.4 under these conditions[13] while that of *p*-nitrophenol[14] is 11.2), and

that the elimination step of eq 2a would determine the rate (assuming reasonably the methanolysis of acetic anhydride to be more rapid that its reversion to **1**). This point remains experimentally unestablished, however.

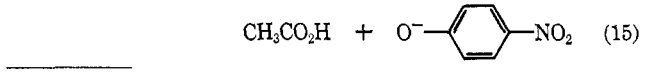
Competition of Nucleophilic and Protolytic Catalysis in Water and Methanol. It is striking that the acetate-catalyzed hydrolysis of *p*-nitrophenyl acetate occurs with nearly equal amounts of the nucleophilic (56–58%) and protolytic (42–44%) modes of catalysis,[5] while the methanolysis reactions is subject to an exclusively nucleophilic type of acceleration. The equilibrium of eq 14, for which the equilibrium constant[15] is k_N/k_P,



thus lies further to the right in methanol (R = CH_3) than in water (R = H).[16] The chief differences in the two cases are likely to arise from (a) the effect of methanol solvent relative to aqueous solvent on the stabilities of **2**, which is intermediate in structure between an acetate ion and an alkoxide ion, and **3**, which is intermediate in structure between an alkoxide ion and *p*-nitrophenoxide ion, (b) the polar effect of CH_3 relative to H, and (c) the steric effect of CH_3 relative to H.

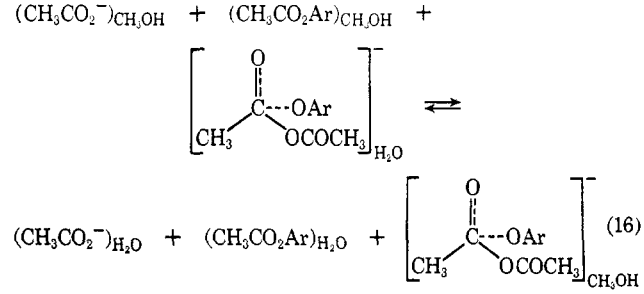
The polar effect of the methyl group cannot be predicted with confidence because it is unknown whether the RO moiety of **2** bears a positive or negative charge relative to ROH (*i.e.*, whether OC-bond formation has proceeded to a greater or smaller extent than OH bond fission in **2**). In the former case, protolytic catalysis is favored in methanol but in the latter case, nucleophilic catalysis. The steric effect of the methyl group clearly favors nucleophilic catalysis by destabilization of **2** in which R exists in a more compressed environment.

The direction of the solvent effect may be estimated by taking **2** as like acetate ion, **3** as like *p*-nitrophenoxide ion, and by using the equilibrium of eq 15 to derive the solvent effect on their relative stabilities. From the pK_a's of acetic acid (4.8 in water[15] and 9.4 in methanol[13]) and *p*-nitrophenol (7.2 in water[17] and 11.2 in methanol[14]), the pK of the reaction of eq 15 is 2.4 in water and 1.8 in methanol. By this criterion, therefore, **3** should be favored over **2** to a greater degree in methanol than in water or in other words, nucleophilic catalysis should dominate protolytic catalysis to a greater degree in methanol, as is observed. This effect can give rise to a factor of no more than four (*i.e.*, $10^{2.4}/10^{1.8}$) in the relative rates.



Our conclusion is that nucleophilic catalysis is favored over protolytic catalysis to a greater degree in methanol than in water for the following reasons. First, there is a smaller spread of anion stabilities in methanol than in water so that a partially formed *p*-nitrophenoxide ion is closer in free energy to a partially formed acetate ion in methanol than in water. Since the activated complex for nucleophilic catalysis (**3**) probably resembles the former while the activated complex for protolytic catalysis (**2**) probably resembles the latter, nucleophilic catalysis will be a better competitor with protolytic catalysis in methanol than in water. Second, nucleophilic catalysis, with a rate-determining activated complex not containing a solvent molecule, will be favored over protolytic catalysis in methanol compared to water because the activated complex for the latter catalytic mode is crowded, contains a solvent molecule and will therefore be destabilized by the bulky methanol. Third, *if* removal of the solvent proton by catalyst in the protolytic activated complex has proceeded to a greater extent than has formation of the bond between the carbonyl group and the solvent oxygen atom, then the electron-donating methyl group will destabilize this activated complex and thus favor nucleophilic catalysis in methanol. Otherwise, the effect will be in the opposite direction.

Nucleophilic Catalysis in Water and Methanol. A comparison of our rate constant for nucleophilic catalysis in methanol at 25° (1.4×10^{-4} M^{-1} sec^{-1}) with that of Oakenfull, Riley, and Gold[5] for the nucleophilic reaction in water at 25° (3.23×10^{-6} M^{-1} sec^{-1}) shows the process to be about 45-fold faster in methanol. The reasons for this solvent effect may be discussed in terms of the equilibrium of eq 16, for which thermodynamic functions have been calculated[17] from our data and those of Oakenfull, Riley, and Gold.[5] The equilibrium constant for eq 16 is $k_N^{CH_3OH}/k_N^{H_2O}$ and thus has a value of about 45.



$\Delta G^\circ = -2.24$ kcal/mol, $\Delta H^\circ = -9$ kcal/mol, $\Delta S^\circ = -23$ eu

(13) R. L. Schowen and K. S. Latham, Jr., *J. Am. Chem. Soc.*, **89**, 4677 (1967).

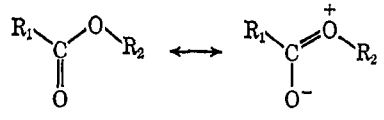
(14) B. W. Clare, D. Cook, E. C. F. Ko, Y. C. Mac, and A. J. Parker, *ibid.*, **88**, 1911 (1966).

(15) Or "virtual equilibrium constant": J. L. Kurz, *ibid.*, **85**, 987 (1963).

(16) It is assumed in writing eq 14 that, as argued above, the elimination step of eq 2a is rate determining in nucleophilic catalysis, and further that the addition step is rate determining in protolytic catalysis, as is portrayed in Scheme II above. The latter point is lent some support by the observation[1] that the methoxide-catalyzed methanolysis of aryl methyl carbonates, labeled with tritium in the methyl group, is much more rapid than exchange of methoxyl groups with solvent. Thus the tetrahedral intermediate for these compounds (assuming a two-step mechanism) expels aryl oxide more rapidly than methoxide, as expected on the grounds of their basicities. Unless positive charge is generated on the leaving groups in the general acid catalyzed eliminations of interest here, it is unlikely that an inversion of the relative leaving group abilities would occur.

(17) From the rate constants, $\Delta G^*_{CH_3OH} = 22.70 \pm 0.04$ kcal/mol and $\Delta G_{H_2O} = 24.94$ kcal/mol. From our temperature-dependence data given in the Results section, $\Delta H^*_{CH_3OH} = 11 \pm 1$ kcal/mol and $\Delta S^*_{CH_3CH} = -39 \pm 3$ eu. Oakenfull, Riley, and Gold[5] give for nucleophilic catalysis, $\Delta S^*_{H_2O} = -16$ eu, whence $\Delta H^*_{H_2O} = 20.2$ kcal/mol. No error limits were cited by these authors in their preliminary communication.[5]

Nucleophilic catalysis in methanol is apparently favored by a considerable enthalpic factor which more than compensates for a large, unfavorable entropy loss. An entropy change of 23 eu is almost certainly connected with changes in entropy of solvation rather than with solvent-induced changes in the entropy of internal degrees of freedom.[18] The implication is then that activation in methanol results in an increase in solvent structure to a greater extent than in water.[19] The increase in structure is probably accompanied by a liberation of enthalpy[20] which may be estimated[1] as $\sim 330\Delta S^\circ$ or about 7–8 kcal/mol. Since the heat-entropy compensation temperature[1,20] of 330° is near the experimental temperature, the solvation effects scarcely appear in ΔG°. The favorable ΔG° of 2.24 kcal/mol can then be ascribed mostly to nonsolvation terms. The simplest is probably the smaller spread of anion stabilities in methanol (see above) which makes conversion of an acetate ion into a partially formed aryloxide ion easier in this solvent than in water. This cannot be the principal origin of the observed 45-fold acceleration because the approximate maximum contribution from this source is given by the ratio of equilibrium constants for eq 15 in water and methanol ($K_{CH_3OH}/K_{H_2O} = 10^{0.6}$), which yields only about four-fold. Another contribution may arise from differences in the effective electrophilicity of the substrate ester carbonyl group in water and in methanol. The delocalization depicted in structure **4** should be more important in the ion-stabilizing solvent water, the



4

ester group should thus be more stable and (since no such forms contribute to the activated-complex structure) less reactive toward addition.

We cannot yet specify why there is a greater increase in solvent structure on activation in methanol than in water. It is not even known whether the activated complex is more or less basic than acetate ion, since it cannot be stated whether it resembles more closely an alkoxide ion or an aryl oxide ion. There is, however, a practical significance to the more negative entropy of activation for nucleophilic catalysis in methanol: the observed ΔS^* of -39 eu is about equal in magnitude to entropies of activation for protolytic catalysis in water while ΔS^* for nucleophilic catalysis in water[5] are about 10–16 eu. Unless protolytic catalysis in methanol[21] yields still more negative entropies of activation, due to inclusion of one or more extra solvent molecules in the activated complex, then the useful entropy criterion ($\Delta S_N^* - \Delta S_P^* \cong 20$ eu) of Oakenfull, Riley, and Gold[5] for distinguishing catalytic mode in water will not apply to methanol.

(18) See L. L. Schaleger and F. A. Long, *Advan. Phys. Org. Chem.*, **1**, 1 (1963), for a discussion of factors contributing to entropies of activation.

(19) Solvent-structural changes on activation in similar reactions will be discussed in a forthcoming paper by C. G. Mitton, M. Gresser, and R. L. Schowen.

(20) (a) L. G. Hepler, *J. Am. Chem. Soc.*, **85**, 3089 (1963); (b) E. M. Arnett and J. J. Burke, *ibid.*, **88**, 4308 (1966); (c) R. L. Schowen, *J. Pharm. Sci.*, **56**, 931 (1967).

(21) Activation parameters are known neither for the protolytically catalyzed ethanolysis of ethyl trifluoroacetate, studied by Johnson,[9] nor for the protolytically catalyzed methanolysis of aryloxysilanes.[13,22]

(22) (a) R. L. Schowen and K. S. Latham, Jr., *J. Am. Chem. Soc.*, **88**, 3795 (1966); (b) K. S. Latham, Jr., and C. Newton, unpublished work.

Experimental Section

***p*-Nitrophenyl acetate** was prepared by allowing 13.9 g (0.1 mol) of *p*-nitrophenol (Matheson Coleman and Bell) and 8 ml (0.11 mol) of acetyl chloride to react in a test tube. The slightly yellow crystals were recrystallized from aqueous ethanol yielding white crystals, mp 76.4–77.4° (lit.[23] 77.5–78°).

Methyl-t_1 acetate ($CH_2TO_2CH_3$) for control experiments was prepared by slowly adding 2 ml (0.04 mol) of methanol-t_1 (CH_2TOH, New England Nuclear Corp.) to 2 ml (0.03 mol) of acetyl chloride. The crude mixture was distilled. A fraction of the distillate collected between 55 and 60° was injected onto a 10 ft × 0.25 in. 16.7% Carbowax 20M on 60–80 Chromosorb W column at room temperature, and the methyl-t_1 acetate collected. The methyl-t_1 acetate was rinsed from the collection tube with methyl acetate.

Methyl acetate-t_1 ($CH_3O_2CCH_2T$) for control experiments and for determination of the effective specific activity of the catalyst was prepared by placing approximately 0.15 g (1.83 mmol) of crushed, diluted sodium acetate-t_1 (NaO_2CCH_2T, New England Nuclear Corp., diluted as described below), 0.3 ml (2.92 mmol) of nitrobenzene (Matheson Coleman and Bell, distilled), and 0.3 ml (3.17 mmol) of dimethyl sulfate (Matheson Coleman and Bell, distilled) in a modified Hickman microstill. The still was placed in an oil bath which was already at 135°. Product was almost immediately observed in the distillate bulb and was shown by integration of the nmr spectra to be approximately 97% pure methyl acetate-t_1 (τ 6.38 and 8.01) with dimethyl sulfate (τ 6.05) as the impurity. The method is akin to that of Stodula, in which dicyclohexylethylamine is employed as solvent.[24]

Buffer Solutions. A 0.4 *M* solution of each of the following was prepared by adding to absolute methanol the stoichiometric amount of the reagent: lithium acetate (Matheson Coleman and Bell), acetic acid (Fischer), lithium chloride (Fischer), and lithium perchlorate (G. Frederick Smith). These stock solutions were then combined and diluted with methanol (Matheson Coleman and Bell) to give the desired acid–salt ratio and base concentration. An ionic strength of 0.1 *M* was maintained by addition of lithium chloride (Fischer) with the exception of several runs in which lithium perchlorate (G. Frederick Smith) was used. Buffers of constant acid–base ratio, prepared in this way, have constant pH.[25]

Kinetic Procedure. *p*-Nitrophenyl acetate was dissolved in the desired buffer solution to give approximately 10^{-4} *M* solution using glass-stoppered volumetric flasks, which were thermostated at 27.4 ± 0.1 or 36.6 ± 0.1° (National Bureau of Standards calibrated thermometer). Samples were withdrawn periodically for ultraviolet analysis. A matched set of glass-stoppered Pyrocell silica cells was used for the measurements with a Cary Model 14 spectrophotometer, Beckman DB spectrophotometer or Beckman DU-2 spectrophotometer. The reaction rates were determined by following the decrease in ultraviolet absorption at 267.5 mμ due to the conversion of *p*-nitrophenyl acetate to methyl acetate and *p*-nitrophenol.

Counting Procedure. Toluene counting solution was prepared by diluting 5 g of PPO (2,5-diphenyloxazole, Packard Instrument Co.) and 0.3 g of POPPO (1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene, Packard Instrument Co.) to 1000 ml with toluene (Mallinckrodt).

An aliquot of 5 ml of the methanol solution to be counted was added to 8 ml of toluene and 8 ml of toluene counting solution in a counting bottle. Background counts were obtained using solutions of 5 ml of methanol, 8 ml of toluene, and 8 ml of toluene counting solutions and were subtracted from sample counts. A Beckman liquid scintillation counter was used in all experiments. The counts obtained by this procedure were proportional to the amount of radioactive substance present within a 1.1% standard deviation.

In order to avoid determination of absolute radioactivities or of corrections for different quenching characteristics, the effective specific molar activity of the catalyst acetate ion was determined by its quantitative conversion to methyl acetate, as described above, and counting of this substance under conditions identical with

(23) M. L. Bender and B. W. Turnquest, *J. Am. Chem. Soc.*, **79**, 1652 (1957).

(24) F. H. Stodula, *J. Org. Chem.*, **29**, 2490 (1964).

(25) K. S. Latham, Ph.D. Thesis in Chemistry, University of Kansas, 1966.

those for counting the product methyl acetate. In this way the constants α of eq 8 for the catalyst and for the product were ensured equal.

Tracer Experiments. Sodium acetate-t_1 (NaO_2CCH_2T) (New England Nuclear Corp.) (61.5 mg, 75 mCi) was diluted 150-fold with sodium acetate (Fisher Certified) by dissolving both in water distilled in glass from $KMnO_4$ and then freeze-drying the mixture. This was accomplished by freezing the solution with Dry Ice–acetone in a thin layer on the sides of a 150-ml, round-bottomed flask, and then allowing the solution to warm slowly to room temperature while a receiving flask was cooled with Dry Ice–acetone and the system continuously pumped.

A methanol solution (25 ml) 0.1 *M* in the diluted sodium acetate-t_1, 0.4 *M* in acetic acid (Fisher Certified), and 0.001 *M* in *p*-nitrophenyl acetate was thermostated in a glass-stoppered flask at 27.4 ± 0.1°. Samples of 5 ml were withdrawn periodically. To the sample was added 1.0 ml of 0.434 *M* hydrochloric acid in methanol to quench the reaction. The sample was frozen using liquid nitrogen as coolant in a thin layer on the sides of a 15 ml, round-bottomed flask attached to an evacuable, two-armed, short-path still. The still was evacuated to a pressure of 0.1 mm and closed off. The sample was allowed to distill to the receiver which contained 0.1800 g (3.34 mmol) of sodium methoxide to neutralize the acetic acid. The distillation was then repeated to separate methyl acetate-t_1 and methanol as the distillate from the sodium acetate just formed by neutralization of the acetic acid. An aliquot of this distillate was counted. The weight of the distillate and the weight of the aliquot of distillate added to the scintillation solution were determined so that the fraction of the distillate counted and the effective activity of the whole distillate could be determined.

Control experiments showed the procedure reproducibly to yield the correct activity of the starting methyl acetate within 4.5%. In the absence of labeled methyl acetate, no radioactivity appeared in the final distillate.

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Glycoside Hydrolysis. III. Intramolecular Acetamido Group Participation in the Specific Acid Catalyzed Hydrolysis of Methyl 2-Acetamido-2-deoxy-β-D-glucopyranoside[1]

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Abstract: The hydrolyses of methyl and pyranosyl glycosides of glucose (Gl) and N-acetylglucosamine (NAG) have been studied in the low pH range at 78.2°, μ = 0.3. The rates of hydrolysis of methyl 2-acetamido-2-deoxy-β-D-glucopyranoside (Me-β-NAG), di-N-acetylchitobiose (NAG_2), methyl β-D-glucopyranoside (Me-β-Gl), cellobiose (Gl_2), and methyl 2-acetamido-2-deoxy-α-D-glucopyranoside (Me-α-NAG) are proportional to hydrogen ion activity (a_H), indicating a specific acid catalyzed mechanism. Me-β-NAG and NAG_2 yield product solutions with $[\alpha]^{30}D$ values equal to that of NAG; Me-β-Gl and Gl_2 yield product solutions with $[\alpha]^{30}D$ values equal to that of Gl. Me-α-NAG hydrolyses with a second-order specific acid catalyzed rate (k_H) one-fourth that of its β anomer, and yields a product solution with an $[\alpha]^{30}D$ value which suggests the formation of a product mixture of methyl 2-amino-2-deoxy-α-D-glucopyranoside and NAG. When log k_H values for β-D-glycosides of NAG are plotted *vs.* the log k_H values for the corresponding β-D-glucopyranosides, a linear relationship is seen to exist for five aryl and pyranosyl glucosides. The point corresponding to the methyl glycosides deviates significantly from the line, and can be accounted for as a 50-fold rate enhancement in the hydrolysis of Me-β-NAG over that anticipated. To account for this rate enhancement two kinetically equivalent mechanisms of hydrolysis of Me-β-NAG are considered: (a) intramolecular general acid catalysis by the protonated 2-acetamido oxygen, and (b) nucleophilic displacement by the 2-acetamido oxygen of the protonated aglycone. The latter mechanism is preferred on the basis of the lower rate of hydrolysis of Me-α-NAG which sterically precludes the possibility of b but not a. Intramolecular acetamido participation in the specific acid catalyzed hydrolysis of Me-β-NAG is concluded to compete favorably with the normal path through an oxocarbonium ion intermediate because the small methyl aglycone does not inhibit the formation of the *trans*-diaxial conformation most favorable to acetamido participation. The significance of this result and its possible relation to the mechanism of lysozyme is discussed.

Lysozyme is the first enzyme to have its tertiary structure determined by X-ray crystallographic methods.[4] From chemical studies[5] and X-ray diffraction studies of complexes of lysozyme with several inhibitors,[6] it is possible to infer that carboxyl groups are the only side-chain functional groups of the enzyme which are both present at the active site and likely to be involved in the bond-breaking steps. Lysozyme specifically hydrolyzes β-linked glycosides of N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM).[7] This fact led to the previous model studies in this series, which demonstrated that the acetamido group of 2-acetamido-2-deoxy-β-D-glucopyranosides assists the spontaneous hydrolysis of the glycoside bond in a stereospecific fashion,[1a] and assists in concert

(1) (a) Part I: D. Piszkiewicz and T. C. Bruice, *J. Amer. Chem. Soc.*, **89**, 6237 (1967); (b) part II: D. Piszkiewicz and T. C. Bruice, *ibid.*, **90**, 2156 (1968).

(2) Predoctoral Fellow, National Institutes of Health. A portion of the material to be submitted by D. P. for the Ph.D. in Chemistry, University of California at Santa Barbara.

(3) To whom inquiries should be directed.

(4) C. C. F. Blake, G. A. Mair, A. C. T. North, D. C. Phillips, and V. R. Sarma, *Proc. Roy. Soc., Ser. B*, **167**, 365 (1967).

(5) J. A. Rupley and V. Gates, *Proc. Natl. Acad. Sci. U. S.*, **57**, 496 (1967).

(6) C. C. F. Blake, L. N. Johnson, G. A. Mair, A. C. T. North, D. C. Phillips, and V. R. Sarma, *Proc. Roy. Soc., Ser. B*, **167**, 378 (1967).

(7) For a summary of known lysozyme substrates, see ref 1b.