compete with one involving an oxocarbonium ion intermediate if a suitable acid catalyst is present and the pyranoside ring undergoes a suitable conformational change. Glutamic acid-35 of lysozyme has been proposed to act as a general acid catalyst[6] and model studies[1b] have demonstrated that a carboxyl group may act as an effective intramolecular general acid catalyst.

In attempting to elucidate the mechanism of catalysis by lysozyme we must answer the question of primary importance, is the 2-acetamido group an absolute requirement for catalysis? A suitable method of answering this question is to examine the interaction of lysozyme with β-glucose oligomers. Since cellobiose is known to bind to lysozyme,[19] larger oligomers should bind more effectively. A determination of K_m and k_{cat} for such oligomers should then demonstrate the significance of the 2-acetamido group in the enzyme mechanism. These experiments are now in progress.

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Absence of Carbonyl Oxygen Exchange Concurrent with the Alkaline Hydrolysis of Substituted Methyl Benzoates[1]

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Abstract: The carbonyl oxygen-18 content of labeled methyl benzoate, methyl *p*-nitrobenzoate, methyl *p*-aminobenzoate, and ethyl benzoate was monitored during the course of alkaline hydrolysis. No oxygen-18 depletion was observed for any of the three methyl benzoates for up to 85% completion of reaction in 33.3% dioxane–water. In addition, the incorporation of solvent oxygen-18 into methyl *p*-nitrobenzoate during alkaline hydrolysis in 33.3% dioxane–water employing water enriched in oxygen-18 was measured. Although the latter results are complicated by possible exchange of oxygen-18 into the *p*-nitro substituent, a minimum value of 36.4 could be assigned to the ratio k_h/k_{ex}. These results are not in agreement with a previously published report of extensive oxygen exchange concurrent with the alkaline hydrolysis of these compounds under these conditions. Both methyl and ethyl benzoate were observed to exchange carbonyl oxygen with solvent oxygen during the course of alkaline hydrolysis in water in the absence of added dioxane. The values of k_h/k_{ex} were 27.7 and 12.6, respectively. These observations are consistent with the interpretation that the principal determinant of the k_h/k_{ex} ratio is the stability of the departing anion; but other factors including the affinity of the anion for acyl carbon as opposed to the proton and rates of proton transfer within the intermediate must be considered in a quantitative evaluation.

Four principal lines of evidence have been advanced in support of the existence of tetrahedral addition intermediates in certain acyl transfer reactions: (1) negative deviations from pH–rate profiles which cannot be attributed to reactant ionization,[3] (2) changes in product distribution after the rate-determining step in the over-all reaction,[4] (3) decreasing slopes in plots of observed rates *vs.* catalyst concentrations,[5] and (4) the existence of concurrent carbonyl oxygen exchange and hydrolysis.[6–8] While the interpretation of the first three of these four criteria in terms of a tetracovalent intermediate has not been challenged, it has been suggested that oxygen-18 exchange might proceed by a mechanism not involving an intermediate.[9] The recent demonstration that oxygen-18 exchange measurements give results entirely consonant with the pH–rate profile for the hydrolysis of ethyl trifluorothiolacetate militates against this latter interpretation at least for this one case.[6]

It has been reported that the ratios of the alkaline hydrolysis rate constants to those for carbonyl oxygen exchange for substituted methyl benzoates are sensitive to the nature of the substituent on the benzene ring. The ratio, measured in 33% dioxane–water, of k_h/k_{ex} ranged from 30 for the *p*-amino compound to 2.8 for the *p*-nitro, and the results were interpreted in terms of the lifetime of the intermediate being of the same order of magnitude as the rate of proton transfer within it.[8] Related work in this laboratory required an ester which would exchange its carbonyl oxygen atom with water in alkaline medium at a rate comparable to the hydrolytic rate. Methyl *p*-nitrobenzoate-carbonyl-

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^{18}O was synthesized for this purpose since the k_h/k_{ex} ratio of 2.8 for this compound is the lowest reported for any organic ester. We report here that we are unable to obtain any evidence for significant carbonyl oxygen exchange concurrent with alkaline hydrolysis in 33.3% dioxane–water, not only with this compound, but also in the case of methyl *p*-aminobenzoate and methyl benzoate which have reported k_h/k_{ex} ratios of 30 and 5.2,[8] respectively.

Experimental Section

Materials. Glass-distilled water was used for all kinetic determinations. Carbon dioxide free water was routinely used for those reactions in which the initial base concentration was less than 0.01 *M*. Oxygen-18 enriched water containing 1.56 or 5.25 atom % ^{18}O was obtained from Yeda Research and Development Co., Ltd., Rehovoth, Israel; water containing 10.25 atom % ^{18}O was obtained from Miles Laboratories, Inc., Elkhart, Ind., and used without further purification. 1,4-Dioxane (Matheson Coleman and Bell, reagent grade) was purified by the method of Fieser[10] with the final distillation performed under nitrogen. The purified material was stored in a dark bottle under positive nitrogen pressure, $n^{25}D$ 1.4189 (lit.[11] $n^{25}D$ 1.4188). Acetonitrile (Matheson Coleman and Bell, reagent grade) was further purified by distillation through a Vigreux column and stored in a dark bottle. All other solvents were reagent grade. Potassium hydroxide solutions were standardized with potassium acid phthalate (National Bureau of Standards, acidimetric standard) and stored in approximately 1.0 *M* solutions in polyethylene bottles. Mercuric chloride (Allied Chemical, reagent grade) was further purified by vacuum sublimation at 180° and stored over anhydrous calcium chloride in a desiccator. 7,8-Benzoquinoline (Aldrich Chemical Co., Inc.) was purified by repeated crystallization from ethanol–water and had a melting point of 50.5–51° (lit.[12] mp 51°).

Unlabeled Compounds. Methyl *p*-nitrobenzoate was prepared by the addition of a 2.4 molar excess of thionyl chloride to a solution of *p*-nitrobenzoic acid in anhydrous methanol at 0°. After removal of solvent the crude product was dissolved in methylene chloride, washed successively with dilute sodium carbonate and water, and crystallized from methanol–water, mp 94–94.5° (lit.[13] mp 93–95°). Methyl benzoate (Eastman White Label) was further purified by distillation through a Vigreux column, and the fraction boiling at 198° was collected, $n^{20}D$ 1.5170 (lit.[14] $n^{20}D$ 1.5170). Ethyl benzoate was prepared by the addition of a 2.8 molar excess of thionyl chloride to a solution of benzoic acid in absolute ethanol at 0°. The solvent was removed at reduced pressure and the remaining solution vacuum distilled. The fraction boiling at 102° (23 mm) was collected, $n^{20}D$ 1.5050 (lit.[14] $n^{20}D$ 1.5058). Methyl *p*-aminobenzoate was prepared from *p*-aminobenzoic acid and anhydrous methanol essentially by the method of Bender and Thomas, mp 111–112° (lit.[8] mp 111°).

Preparation of Oxygen-18-Labeled Compounds. In a typical synthesis oxygen-18-containing *p*-nitrobenzoic acid was prepared by addition of 6.0 ml of 5.25 atom % ^{18}O water (333 mmol) from a pressure-equalizing funnel to a solution of 6.0 g (32.3 mmol) of *p*-nitrobenzoyl chloride (Eastman White Label) in 35 ml of cold dioxane. After the addition of water was completed approximately 20 ml of additional dioxane was added to make the solution homogeneous. The solution was then refluxed for about 24 hr in a system protected from atmospheric water. The solvent was removed at reduced pressure and the crude *p*-nitrobenzoic acid was dried in a vacuum desiccator over anhydrous calcium chloride and phosphorus pentoxide. The methyl ester was prepared by the addition of a 2.0 molar excess of thionyl chloride to a suspension of 6 g of the oxygen-18-containing acid in anhydrous methanol. The product was isolated as described for the ^{16}O compound. A total of 5.3 g (91% yield) was obtained, mp 94.5–95° (lit.[13] mp 93–95°). Oxygen-18 analysis of the *p*-nitrobenzoic acid used in the synthesis gave a value of 2.77 ± 0.01 atom % excess ^{18}O; whereas the ester analysis gave a value of 1.41 ± 0.01 atom % excess ^{18}O demonstrating that oxygen-18 is located uniquely in the carbonyl oxygen positions since only one of the two equivalent carbonyl group oxygen atoms in the acid should be retained in the ester. A second preparation of this ester in which the *p*-nitrobenzoyl chloride was decomposed with a molar equivalent of 5.25 atom % ^{18}O water gave *p*-nitrobenzoic acid containing 1.32 ± 0.01 atom % excess ^{18}O with the methyl ester containing 0.658 ± 0.005 atom % excess ^{18}O.

Oxygen-18-containing benzoic acid was prepared by addition of a 5.75 molar excess of 5.25 atom % ^{18}O water from a pressure equalizing funnel to a solution of benzoic acid in cold dioxane. The solution, containing two drops of concentrated sulfuric acid and protected from atmospheric moisture, was refluxed for about 72 hr. The solvent was removed at reduced pressure and the crude benzoic acid was dried in a vacuum desiccator over anhydrous calcium chloride. Methyl benzoate containing oxygen-18 was prepared by the addition of a 2.0 molar excess of thionyl chloride to a suspension of 5 g of oxygen-18-containing benzoic acid in anhydrous methanol at 0°. After the solution had refluxed overnight the methanol was removed by distillation. The remaining ester was dissolved in methylene chloride and washed with dilute sodium carbonate and water. The methylene chloride was removed at reduced pressure and methyl benzoate obtained by vacuum distillation. A yield of 4.4 g (91%) was obtained: bp 63° (3 mm) (lit.[14] bp 85° (10 mm)) $n^{20}D$ 1.5160 (lit.[14] $n^{20}D$ 1.5170). Analysis of oxygen-18 gave 1.17 ± 0.01 atom % excess ^{18}O in the ester. Ethyl benzoate containing oxygen-18 was prepared by refluxing a solution of 10 g of oxygen-18 benzoic acid in 100 ml of acidic absolute ethanol for 48 hr. Absolute ethanol containing 5% hydrogen chloride was prepared by bubbling dry hydrogen chloride gas into the ethanol. The solvent was removed at reduced pressure and ethyl benzoate was obtained by vacuum distillation. A total of 9.3 g (88.5%) of ethyl benzoate containing 1.38 ± 0.01 atom % excess ^{18}O was obtained: bp 78° (3.5 mm) (lit.[14] bp 90° (10 mm)); $n^{20}D$ 1.5050 (lit.[14] $n^{20}D$ 1.5058). A significant discrepancy between observed and theoretical values for the atom % excess ^{18}O in those acids prepared from the 5.25% oxygen-18 water was observed throughout these studies. In the case of the unsubstituted benzoic acids this is accounted for by the fact that the oxygen-18-enriched water, when analyzed by the method of Boyer,[15] contained 4.49 rather than 5.25 atom % oxygen-18. The situation for nitrobenzoic acid was more complicated. Even after allowance for the lower atom % oxygen-18 content of the water, the atom % oxygen-18 content for the acid was found to be only 79% of theoretical. However, when the methyl ester prepared from this acid was analyzed by mass spectrometry the ratio $[(P + 2)/P]100$ was observed to be 7.08 ± 0.25,[16] where $P + 2$ represents the value of the mass spectral peak corresponding to the molecular ion resulting from molecules of mass 183 and, P, the value of the peak for the parent molecular ion of mass 181. The oxygen-18-enriched acid used in this synthesis when analyzed by conversion to CO_2[17] contained 3.32 atom % oxygen-18. The theoretical value of the $[(P + 2)/P]100$ ratio for the methyl ester prepared from this acid is only 4.51. Thus it appears that in acid solution oxygen-18 is incorporated into other positions in addition to the carbonyl oxygen, *i.e.*, the nitro group. That these oxygen atoms do not appear in CO_2 produced upon pyrolysis is demonstrated by the fact that the theoretical 50% reduction in oxygen-18 content of the CO_2 obtained from pyrolysis was observed when the acid was converted to ester. Previous investigators have reported that neither nitromethane[18] nor nitrobenzene[18,19] exchange oxygen with $H_2{}^{18}O$ in the presence of either acid or alkali. Oxygen-18 containing methyl *p*-aminobenzoate was prepared according to the method of Bender and Thomas[8] using 5.25 atom % ^{18}O water. A yield of 8.9 g (81%) of methyl *p*-aminobenzoate containing 0.346 ± 0.002 atom % excess ^{18}O was obtained from 10 g of *p*-aminobenzoic acid, mp 111–111.5° (lit.[8] mp 111°).

Apparatus. A Zeiss PMQ II spectrophotometer equipped with a thermostated brass cuvette holder, through which water of constant temperature was circulated, was used for all kinetic determinations.

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Difference and product spectra were obtained on a Cary Model 15 recording spectrophotometer. Gas chromatography was performed on an Aerograph Model A-100 gas chromatograph (Wilkens Instrument and Research, Inc.) equipped with a thermal conductivity detector. The oxygen-18 content of the CO_2 obtained from pyrolyzed samples was determined on either an Atlas-Mat Model M86 mass spectrometer, a C.E.C. Model 21-130 mass spectrometer (Consolidated Electrodynamics Corp.), or a C.E.C. Model 21-614 residual gas analyzer modified with an inlet for batch sample analysis. Mass spectral analyses of methyl *p*-nitrobenzoate were performed on a C.E.C. Model 21-103c instrument modified to incorporate an ion multiplier.

Preparation of Samples for Oxygen-18 Analysis. Samples were converted to carbon dioxide by heating with mercuric chloride at 400° for 2 hr.[17] A 14 × 1.5 cm column packed with 7,8-benzoquinoline was routinely used to remove the pyrolytic by-product, hydrogen chloride. Solid compounds were recrystallized to literature melting points before pyrolysis. Methyl and ethyl benzoate were purified by gas chromatography on a 5 ft × $^{1}/_{8}$ in. i.d. stainless steel column packed with 5% Apiezon L (Apiezon Products Ltd.) on Fluropak 80, nonpolar (Wilkens Instrument and Research, Inc.) at 85° with a helium flow rate of 75 cc/min. The liquid samples were transferred to the pyrolysis tube either by means of a microliter pipet or in Microcap disposable pipets (Drummond Scientific Co.) which remained in the pyrolysis tube. In order to keep the liquid from boiling out of the evacuated tube the tip of the tube containing sample was immersed in an isopropyl alcohol–Dry Ice bath immediately prior to evacuation. The reproducibility of the pyrolytic method was ±1–3% as indicated by pyrolysis of a series of identical samples of each compound that was analyzed. The atom % excess oxygen-18, X, was determined by measuring the mass 46/mass 44 ratio and calculated from the relationship

$$X = \left[\frac{R}{2(1 + R)}\right]100 - Q \qquad (1)$$

where R is the ratio mass 46/mass 44 and Q the per cent natural abundance of oxygen-18 which was determined independently for each experiment.

The ratio of the rate constants for hydrolysis and exchange was calculated from the slope of the plot of log $(100E/E_0)$ *vs.* log $(100 \cdot X/X_0)$ where E/E_0 represents the fraction of remaining ester after partial hydrolysis and X/X_0 is the fraction atom per cent excess oxygen-18 in the remaining ester.[20]

A possible source of error in the determination of mass 46/mass 44 ratios could result from artifactual production of molecular ions other than CO_2^+ with m/e ratios of 44 or 46. Pyrolysis of methyl *p*-nitrobenzoate in particular could lead to anomalous values for the ratio mass 46/mass 44 through the production of NO_2^+. In order to test this possibility samples of methyl *p*-nitrobenzoate enriched in oxygen-18 and of natural abundance were pyrolyzed under identical conditions varying the $HgCl_2$/ester (w/w) ratio over a 100-fold range. At $HgCl_2$/ester ratios of 10 or less there was a random variation in the observed atom per cent oxygen-18. This value varied between 0.2020 and 0.3233 for the unenriched ester. No significant variation in the atom per cent oxygen-18 value was observed for samples pyrolyzed at a $HgCl_2$/ester ratio of 100. Under this condition of pyrolysis 88% of the samples analyzed gave values of atom per cent oxygen-18 that were within 1% of the accepted value of 0.2039. The highest value observed for these conditions was 0.2296 ± 0.003 atom % oxygen-18. The oxygen-18-enriched samples showed no significant deviation at high $HgCl_2$/ester ratios. All of the data reported in the Results came from samples pyrolyzed at a $HgCl_2$/ester (w/w) ratio of approximately 100. Additionally, prior to pyrolysis each sample of recovered ester or acid was divided into three identical samples, which were independently pyrolyzed. The values reported represent the weighted average of the grouped data obtained from the three samples. As a result of these precautions and the observation that the maximum nonspecific enrichment obtained at high w/w ratios of $HgCl_2$/ester was only approximately 0.02 atom %, it is considered highly unlikely that significant errors have been introduced by unexpected molecular ions of m/e 44 or 46.

Kinetics of Hydrolysis. Pseudo-first-order rate constants were determined essentially by the method of Jencks and Carriuolo.[21] The alkaline hydrolysis of methyl *p*-nitrobenzoate in 33.3% acetonitrile–water and in 33.3% dioxane–water was monitored by the absorption decrease at 258 and 256 mμ, respectively. The alkaline hydrolyses of methyl benzoate, methyl *p*-aminobenzoate, and ethyl benzoate were followed by the absorption decrease at 282, 296, and 282 mμ, respectively. Two methods were employed for determination of second-order rate constants. The first method consisted of withdrawing 0.1-ml aliquots from the reaction solution at appropriate time intervals and adding the sample to either a 2.0 or a 5.0 ml solution of 33.3% dioxane in 0.01 *M* succinate buffer pH 5.6. With the aid of optical spacers the decrease in absorption was determined at the wavelength reported for pseudo-first-order determinations. Alternatively, the reaction rate could be determined without the use of optical spacers by observing the absorption increase at longer wavelengths. Good agreement between rate constants was obtained for both methods of determination. In all cases the rate constants were obtained by utilizing the appropriate integrated rate equation.

Kinetics of Oxygen Exchange. Samples of the carbonyl oxygen-18-containing esters were partially or "completely" hydrolyzed under conditions exactly identical with those reported for the determination of second-order rate constants. After the appropriate time interval the unreacted ester or acid product of the hydrolysis was recovered. Unreacted methyl *p*-nitrobenzoate was isolated from solutions of ionic strength 0.01 as described by Bender and Thomas[8] except that 0.1 *M* phosphate buffer pH 6.3 rather than pH 7.0 was used to quench the solution. All other reaction mixtures were quenched at the appropriate time by adding the sample to a slight excess of 0.01 *M* hydrochloric acid. The uncorrected pH of the solutions after quenching was always between 6.5 and 7.0. Esters were immediately recovered from the aqueous phase by methylene chloride extraction. After washing the methylene chloride extract with dilute sodium carbonate and water, solid compounds were recovered by removal of methylene chloride at reduced pressure and repeated recrystallization. For the liquid compounds the washed methylene chloride was rapidly dried with anhydrous sodium sulfate which was immediately removed by filtration on a fine porosity glass filter. The methylene chloride solution was then concentrated by passing a stream of dry nitrogen over the surface and the pure ester was recovered after gas chromatography as described. Substituted benzoic acid samples were recovered by adjusting the uncorrected pH of the solution to approximately 1 and extracting the acid from the aqueous phase with methylene chloride. The methylene chloride was removed at reduced pressure and the pure acids were obtained after repeated recrystallization.

Partial hydrolysis of methyl *p*-nitrobenzoate was performed in 33.3% dioxane–oxygen-18-enriched water under conditions identical with those reported for the determination of second-order rate constants at ionic strength 0.01. Water of known oxygen-18 content was prepared by diluting water containing 10.26 atom % oxygen-18 with either water of natural abundance oxygen-18 or water containing 1.56 atom % oxygen-18. The ester recovered after partial hydrolysis was analyzed by mass spectrometry. The carbon dioxide obtained by pyrolysis of the same samples with $HgCl_2$ was also analyzed. The oxygen-18 content of the molecule was monitored by changes in the ratio $[(P + 2)/P]100$. The ratio of the hydrolysis to exchange rate constants was calculated from the slope of the plot of

$$\log (100X/X_0) = k_{ex}/k_h \log (100E/E_0) \qquad (2)$$

where E/E_0 represents the fraction of remaining ester after partial hydrolysis and X/X_0 is that fraction of the remaining ester that has *not undergone* oxygen exchange with solvent oxygen-18.

Results

The second-order rate constants for the alkaline hydrolyses of *para*-substituted methyl benzoates and of ethyl benzoate are reported in Table I. A Hammett plot of the data for solvolysis of the methyl benzoates in 33.3% dioxane–water at ionic strength 0.5 is linear with a ρ value of 1.89. This value of ρ is in excellent agreement with the figure of 1.93 reported for this series of esters in the same solvent at lower ionic strength.[8] The second-order rate constant determined for the alkaline hydrolysis of methyl *p*-nitrobenzoate in 33.3% dioxane–water at ionic strength 0.01 is signif-

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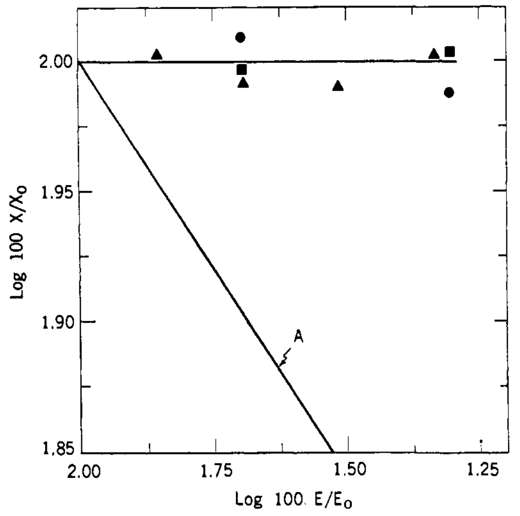

Figure 1. Extent of carbonyl oxygen exchange as a function of extent of alkaline hydrolysis of methyl *p*-nitrobenzoate: ▲, ■, ●, identical experiments performed on different days in 33.3% dioxane–water at 25°; initial [ester] = initial [OH$^-$] = 0.01 *M*, ionic strength 0.01. Line A is calculated from literature data reported for these conditions.[8]

icantly higher than that reported previously.[8] The rate constants obtained in the present study at ionic strength 0.5 for three *para*-substituted methyl benzoates are in rather close quantitative agreement with the previous results obtained at low ionic strength. Because of these observations, the carbonyl oxygen exchange reactions for methyl benzoate and methyl *p*-nitrobenzoate were investigated at both high and low ionic strength and methyl *p*-nitrobenzoate was studied in 33.3% acetonitrile–water as well as in 33.3% dioxane–water.

Table I. Second-Order Rate Constants for the Alkaline Hydrolysis of *para*-Substituted Benzoic Acid Esters[a]

Benzoate	Solvent	No. of determinations	k_{hyd}, M^{-1} min^{-1}
Methyl *p*-nitro-	*c*	8	25.3 ± 0.2[b]
Methyl *p*-nitro-	*d*	5	70.6 ± 3.4
Methyl *p*-nitro-	*e*	4	40.4 ± 2.7
Methyl	*e*	5	1.39 ± 0.08
Methyl	*d*	3	1.60 ± 0.02
Methyl	*f*	4	4.72 ± 0.07
Methyl *p*-amino-	*e*	3	0.0760 ± 0.002
Ethyl	*f*	4	2.15 ± 0.10

[a] Temperature 25.0 ± 0.1°, ionic strength maintained with KCl. [b] Standard deviation. [c] 33.3% acetonitrile–water, ionic strength 0.3. [d] 33.3% dioxane–water, ionic strength 0.01. [e] 33.3% dioxane–water, ionic strength 0.5. [f] Water, ionic strength 0.003.

The oxygen-18 content of methyl *p*-nitrobenzoate-carbonyl-^{18}O was monitored during alkaline hydrolysis in 33.3% dioxane–water at ionic strength 0.01 and the results are shown in Figure 1. No diminution of the oxygen-18 content was observed up to an extent of hydrolysis of 80%. This result disagrees with the previously published figure of k_h/k_{ex} equal to 2.8 (Figure 1, line A) obtained under the same conditions.[8]

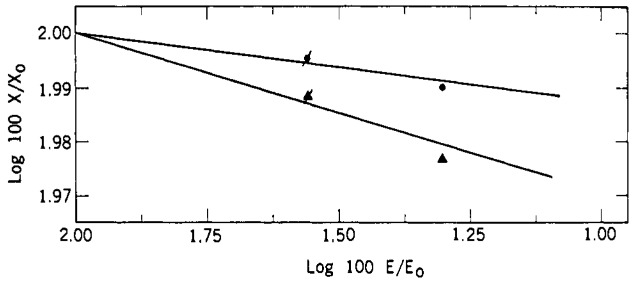

Figure 2. The incorporation of oxygen-18 into methyl *p*-nitrobenzoate from enriched water during alkaline hydrolysis. Conditions are the same as in Figure 1: ▲, oxygen-18 excess of the intac ester molecule determined from the ratio $(P + 2)/P$ where P is th molecular weight of the parent molecular ion; ●, oxygen-18 exces of the CO$_2$ obtained from pyrolysis of the same sample; ▲, ●, identical values obtained from duplicate experiments. The ratio X/X_0 here represents the fraction of remaining ester of natural isotopic abundance.

The lack of a large oxygen exchange reaction during the alkaline hydrolysis of this compound was further demonstrated by experiments in which ester of natural isotopic abundance was partially hydrolyzed in isotopically enriched water and the isolated ester examined directly without pyrolysis in a mass spectrometer.[22a] A small but definite increase in the ratio $(P + 2)/P$ as a function of extent of alkaline hydrolysis of this compound was observed (Table II) and the results are plotted according to eq 2 as the lower line in Figure 2. The ratio k_h/k_{ex} calculated from this line is equal to 36.4. This figure is also very much higher than the published value and thus confirms the experiments done with labeled ester.[22b] It is probable that the true ratio of k_h/k_{ex} is considerably greater than 36.4 since the CO$_2$ obtained from the pyrolysis of the samples listed in Table II contained only about 40% of the excess oxygen-18 of the parent ester. These results are plotted as the upper line in Figure 2 and give

Table II. Incorporation of Oxygen-18 into Methyl *p*-Nitrobenzoate during Hydrolysis in 33.3% Dioxane–Oxygen-18-Enriched Water at 25° and Ionic Strength 0.01

Expt no.	Completion of hydrolysis, %	$[(P + 2)/P]_f/[(P + 2)/P]_0$[a] predicted for condition of carbonyl oxygen exchange[b]	$[(P + 2)P]_f/[(P + 2)P]_0$ obsd
	0	1.00	1.00
1[c]	63.4	2.09	1.09 ± 0.05
2[c]	63.4	2.09	1.09 ± 0.05
3[d]	80.0	2.55	1.16 ± 0.04
	100.0	5.04,[c] 4.64[d]	

[a] $[(P + 2)/P]_f$ is the ratio of the heights of peak 183 to peak 181 at the indicated fractional completion of hydrolysis. $[(P + 2)/P]_0$ denotes the same ratio at zero per cent hydrolysis. [b] Values calculated from the relationships $k_h/k_{ex} = \log (E/E_0)/\log (X/X_0)$ where the ratio X/X_0 represents the fraction of remaining ester of natural isotopic abundance and the published value of $k_h/k_{ex} = 2.8$. Only the incorporation of label into the carbonyl oxygen position was considered in these calculations. The discrepancy between observed and calculated values would be even larger if the exchange into the *p*-nitro substituent were included. [c] Hydrolysis was performed in 4.79 atom % oxygen-18 water. [d] Hydrolysis was performed in 4.34 atom % oxygen-18 water.

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(22) (a) This experiment was suggested by Drs. F. J. Kezdy and M. L. Bender. (b) Drs. D. VanderJagt and M. L. Bender have independently done this experiment and found $k_h/k_{ex} \geq 50$ (personal communication).

Table III. Comparison of Observed and Calculated Values of Atom Per Cent Excess ^{18}O in Recovered Compounds

Reaction conditions	Completion of hydrolysis, %	Compd recovered	Excess ^{18}O in ester before hydrolysis, atom %	Excess ^{18}O in recovered compd,[a] atom %	Excess ^{18}O predicted for condition of depletion,[b] atom %
		Methyl *p*-Nitrobenzoate			
33.3% dioxane, $\mu = 0.5$	80	Ester	1.41 ± 0.03	1.36 ± 0.1	0.795
[ester] = 0.01 *M*, $[OH^-]$ = 0.01 *M*	80	Ester	1.51 ± 0.01	1.57 ± 0.02	0.855
33.3% dioxane, $\mu = 0.5$	80	Ester	1.41 ± 0.03	1.46 ± 0.07	0.795
[ester] = 0.01 *M*, $[OH^-]$ = 0.02 *M*	90	Ester	1.51 ± 0.01	1.57 ± 0.02	0.667
33.3% dioxane, $\mu = 0.5$	80	Ester	1.41 ± 0.03	1.42 ± 0.06	0.795
[ester] = 0.01 *M*, $[OH^-]$ = 0.03 *M*	95	Ester	1.51 ± 0.01	1.76 ± 0.05	0.517
33.3% dioxane, $\mu = 0.1$	>99	Acid	1.35 ± 0.03	1.38 ± 0.09	0.994
[ester] = 0.01 *M*, $[OH^-]$ = 0.01 *M*	>99	Acid	0.638 ± 0.01	0.644 ± 0.07	0.470
33.3% dioxane, $\mu = 0.5$	>99	Acid	1.35 ± 0.03	1.42 ± 0.05	0.994
[ester] = 0.01 *M*, $[OH^-]$ = 0.01 *M*					
33.3% acetonitrile, $\mu = 0.3$	>99	Acid	1.35 ± 0.03	1.43 ± 0.05	(0.994)[c]
[ester] = 0.01 *M*, $[OH^-]$ = 0.02 *M*					
		Methyl Benzoate			
33.3% dioxane, $\mu = 0.5$	>99	Acid	1.069 ± 0.006	1.100 ± 0.01	0.890
[ester] = 0.01 *M*, $[OH^-]$ = 0.02 *M*	>99	Acid	1.069 ± 0.006	1.102 ± 0.03	0.890
33.3% dioxane, $\mu = 0.01$	>99	Acid	1.24 ± 0.02	1.25 ± 0.008	1.055
[ester] = 0.01 *M*, $[OH^-]$ = 0.01 *M*	>99	Acid	1.24 ± 0.02	1.25 ± 0.003	1.055

[a] Values reported are the weighted averages for grouped data. Standard deviations are calculated from the unweighted mean. [b] Atom per cent excess calculated for recovered esters from the relation $k_h/k_{ex} = \log (E/E_0)/\log (X/X_0)$ and reported values of k_h/k_{ex} in 33.3% dioxane–water.[8] Atom per cent excess calculated for recovered acids from the relation $(R/R_0)_f = k_h/(k_h + k_{ex})$ which is readily derived from the relationship reported by Bender and d'A. Heck[6] when the carboxylic acid product does not undergo exchange with solvent. [c] Value calculated for exchange in 33.3% dioxane–water.

a value of k_h/k_{ex} of 80. The difference may be due to a small hydroxide ion catalyzed exchange of the oxygen atoms in the *p*-nitro substituent which are not converted to CO_2 by $HgCl_2$ at 400°. These oxygen atoms apparently exchange readily with solvent in acid (Experimental Section).

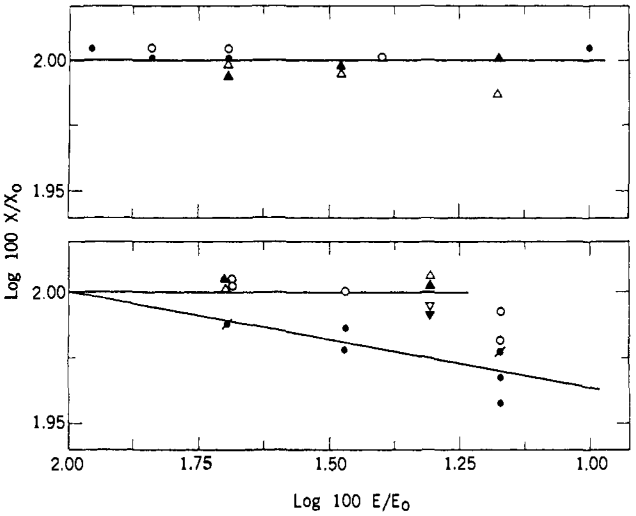

Figure 3. Carbonyl oxygen-18 content of esters as a function of the degree of alkaline hydrolysis at 25°: top ○, ●, methyl *p*-nitrobenzoate, duplicate experiments in 33.3% acetonitrile–water, initial [ester] = 0.01 *M*, initial $[OH^-]$ = 0.02 *M*, ionic strength 0.3; △, ▲, methyl *p*-aminobenzoate, duplicate experiments in 33.3% dioxane–water, initial [ester] = 0.02 *M*, initial $[OH^-]$ = 0.06 *M*, ionic strength 0.5; bottom △, ▲, ▽, ▼, methyl benzoate, identical experiments performed on different days in 33.3% dioxane–water, initial [ester] = 0.01 *M*, initial $[OH^-]$ = 0.02 *M*, ionic strength, 0.5; ○, in 33.3% dioxane–water, initial [ester] = 0.01 *M*, initial $[OH^-]$ = 0.01 *M*, ionic strength 0.01; ●, in water, initial [ester] = 0.003 *M*, initial $[OH^-]$ = 0.003 *M*, ionic strength 0.003; ●̸, identical values from duplicate experiments.

Further attempts to measure carbonyl oxygen-18 depletion during the course of alkaline hydrolysis of methyl *p*-amino-, *p*-nitro-, and *p*-H benzoates under various conditions of organic solvent and ionic strength are shown in Figure 3. No oxygen exchange was observed for any of these esters in dioxane–water. The reported values of k_h/k_{ex} for methyl benzoate and methyl *p*-aminobenzoate are 5.2 ± 0.8 and 30 ± 4, respectively, in 33.3% dioxane–water.[8] Some exchange was observed during the hydrolysis of methyl benzoate in the absence of added organic solvent. The value of k_h/k_{ex} under these conditions was 27.7.

Other data representing single point kinetic determinations are collected in Table III. In these experiments unreacted esters or the carboxylic acid products were isolated after partial or complete hydrolysis, respectively, and the observed oxygen-18 content of the recovered compounds is compared with starting ester and with the expected values calculated from the reported ratios of k_h/k_{ex}.[8] In no case was any evidence for oxygen-18 exchange obtained.

Because of the discrepancies between the results reported above and the literature, it was considered desirable to reinvestigate the oxygen-18 exchange kinetics of ethyl benzoate. These experiments are shown in Figure 4. The value of k_h/k_{ex} at 25° was equal to 12.6. This result, obtained in the absence of added organic solvent, may be compared with the previously published figures of 11.3[23] and 10.6[20] obtained in 33.3% dioxane–water and 4.8[20] in water.

Discussion

The data presented demonstrate that no significant exchange of carbonyl oxygen with solvent occurs during the course of alkaline hydrolysis of methyl benzoate, *p*-nitrobenzoate, or *p*-aminobenzoate in 33.3% dioxane–

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(23) M. L. Bender, R. D. Ginger, and J. P. Unik, *J. Amer. Chem. Soc.*, 80, 1044 (1958).

Table IV. Estimates of k_h/k_{ex} for the Reactions of Substituted Benzoic Acid Esters in Alkaline Solution at 25°

Benzoate	Solvent	k_{ex}/k_h[a]	No. obsd	k_h/k_{ex}[b]	95% confidence intervals[c]
Methyl *p*-nitro-	*d*	0.0107 ± 0.004	10	93	>55
Methyl *p*-nitro-	*e*	−0.0135 ± 0.004	9	*h*	∞[i]
Methyl	*f*	−0.010 ± 0.006	4	*h*	>132
Methyl	*d*	0.0112 ± 0.006	5	89	>40
Methyl	*g*	0.0361 ± 0.003	8	27.7	23–35
Methyl *p*-amino-	*f*	0.0073 ± 0.003	6	137	>84
Ethyl	*g*	0.0795 ± 0.003	6	12.6	11.4–14.0

[a] Values and standard errors were determined from a linear regression of log $(100X/X_0)$ on log $(100E/E_0)$ forced through the coordinates (2.0, 2.0). The errors in the latter quantity were assumed to be vanishingly small compared to those in the former for the purpose of constructing the regression line: E. J. Williams "Regression Analysis," John Wiley and Sons, Inc., New York, N. Y., 1959, p 15. [b] Most probable value; *i.e.*, the reciprocal of the regression coefficient given in column 3. [c] Obtained from the appropriate "one-tailed" or "two-tailed" value of t and the formula $t = b_1 - \beta_1/\mathrm{SE}b_1$ where b_1 is the regression coefficient, $\mathrm{SE}b_1$ the estimated standard error in b_1, and β_1 the specified value being tested: N. Draper and H. Smith "Applied Regression Analysis," John Wiley and Sons, Inc., New York, N. Y., 1966, p 19. [d] 33.3% dioxane–water, ionic strength 0.01. [e] 33.3% acetonitrile–water, ionic strength 0.30. [f] 33.3% dioxane–water, ionic strength 0.50. [g] Water, ionic strength 0.003. [h] Negative regression coefficient, most probable value >100. [i] This value is a result of the negative regression coefficient which may be caused by isotope discrimination against carbonyl-^{18}O in the hydrolysis reaction (see text).

water. This result was repeatedly obtained for all three esters under conditions identical with those for which extensive oxygen exchange has previously been reported to occur.[8] The data of Figure 3 and Table III indicate that the lack of oxygen exchange in these esters is not, a function of the ionic strength of the hydrolysis solution and is independent of the initial base-to-ester ratio as well as the substitution of acetonitrile for dioxane as the organic component of the solvent. We are unable to reconcile these results with those previously published. The question of the occurrence of oxygen exchange is one of whether the slope of the lines passing through 2.00 on the ordinate of Figures 1–4 is experimentally less than zero. Visual inspection of Figures 1 and 3 reveals that the only methyl ester for which this result was obtained was methyl benzoate when hydrolyzed in water. Bender, *et al.*, have proposed a relationship to provide an estimate of the lower limit for k_h/k_{ex}.[24] Their procedure consists of using the average deviation in the oxygen-18 determinations as a measure of the possible oxygen exchange in the sample isolated at the highest per cent hydrolysis. An alternative approach to the problem of estimating limits for the values of k_h/k_{ex} is through the construction of the least-squares line forced through the coordinates (2.00, 2.00) of Figures 1, 3, and 4, with log $(100X/X_0)$ treated as the dependent and log $(100E/E_0)$ as the independent variable. The slope of the regression line is then equal to the most probable value of k_{ex}/k_h. These figures and the associated standard errors are given in Table IV. Confidence levels determined by a standard t test show that of the reactions investigated only the hydrolysis reactions of methyl benzoate in the absence of organic solvent and of ethyl benzoate are accompanied by a significant amount of carbonyl oxygen exchange. The values of k_h/k_{ex} for the remaining cases listed in Table IV are all >40 and in some cases are most likely >100. More precise estimates of high values of k_h/k_{ex} are precluded by the practical limitation that the standard errors of the estimates of k_{ex}/k_h remain relatively constant as the absolute values of this ratio approach zero. In several of the experiments connected with this study small but definite *increases* with time in the carbonyl-^{18}O content of the esters were noted (Tables III and IV), possibly indicating preferential hydrolysis of the ^{16}O over the ^{18}O ester. To the extent that such a selectivity does obtain, values of k_h/k_{ex} for esters that do exchange carbonyl oxygen would be high and the errors more serious for the higher values of this ratio assuming a constant ^{18}O isotope effect.

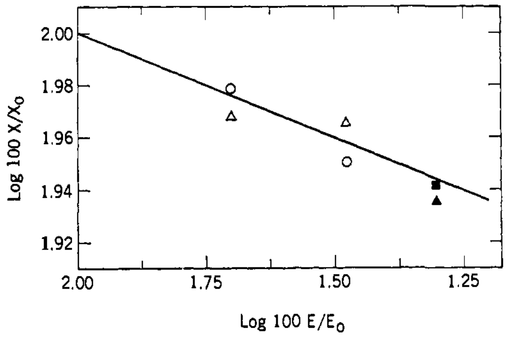

Figure 4. Extent of carbonyl oxygen exchange as a function of extent of alkaline hydrolysis of ethyl benzoate: ○, △, ■, ▲, identical experiments performed on different days in water at 25°, initial [ester] = initial [OH$^-$] = 0.003 or 0.004 *M*.

Determinants of Carbonyl Oxygen Exchange Rates. It is clear from the results reported here that the substitution of an ethyl for a methyl group in the alcohol moiety of esters of benzoic acid is responsible for a large increase in k_h/k_{ex}. Isopropyl and *t*-butyl benzoates are also reported to exchange carbonyl oxygen during the course of alkaline hydrolysis,[20] whereas various substituted benzyl benzoates[24] and phenyl benzoate[25] do not. The fact that substituted methyl benzoates are now known to exchange carbonyl oxygen with solvent less readily than ethyl benzoate allows the exchange rate to be at least roughly correlated with the relative stabilities of the departing anions as compared to hydroxide ion.

The value of k_h/k_{ex} obtained for the reaction of ethyl benzoate in water at low ionic strength was 12.6 while that of methyl benzoate under identical conditions was 27.7. The pK_a values of methanol and ethanol are 15.5 and 16, respectively.[26] Thus the logarithms of the

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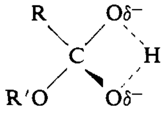
(24) M. L. Bender, H. Matsui, R. J. Thomas, and S. W. Tobey, *J. Amer. Chem. Soc.*, **83**, 4193 (1961).

(25) C. A. Bunton and D. N. Spatcher, *J. Chem. Soc.*, 1079 (1956).

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

$k_{\mathrm{h}}/k_{\mathrm{ex}}$ ratios are approximately dependent upon the first power of the pK_{a} of the conjugate acid of the leaving alkoxide ion for at least these two esters. Benzyl alcohol is approximately as acidic as methanol in isopropyl alcohol solution[27] and consequently benzyl benzoate might be expected to undergo a significant oxygen-exchange reaction in the absence of organic solvent. This reasoning implies in terms of a tetrahedral addition intermediate that exchange will be observed only when the rate of breakdown to give hydroxide ion and starting material is comparable with or slower than that of product formation, assuming fast proton transfer within the intermediate. Quantitatively, however, this explanation is inadequate because it would predict that $k_{\mathrm{h}}/k_{\mathrm{ex}}$ would be less than 1 for ethyl benzoate and only slightly greater than 1 for methyl benzoate given the pK_{a} of 15.7 for water. Any of three explanations might be offered in attempting to account for ratios of $k_{\mathrm{h}}/k_{\mathrm{ex}}$ that are higher than those predicted from the relative stabilities of the leaving alkoxide ion compared to OH^-. The first is the possibility of incomplete proton transfer within the intermediate, *i.e.*, the lifetime of the tetrahedral intermediate is comparable with or shorter than the time required to attain proton equilibrium. This rationalization has been offered previously[8] and is supported by the fact that $k_{\mathrm{h}}/k_{\mathrm{ex}}$ is increased by two- to threefold in the neutral hydrolysis of ethyl trifluoroacetate when the reaction is run in D_2O.[6] Unfortunately this result from a neutral hydrolysis reaction does not permit any conclusions with respect to alkaline hydrolysis where an anionic rather than neutral transition state would be formed. The fact that the ratios of $k_{\mathrm{h}}/k_{\mathrm{ex}}$ for the reactions of ethyl and methyl benzoates in base are sharply increased upon addition of dioxane[28] can be interpreted either in terms of the stability of the departing anion in the solvent or of more molecules of water being involved in the exchange than in the hydrolysis reaction. However, the $\Delta S^{\ddagger}$ for both the hydrolysis and exchange reactions of ethyl benzoate are very close to -28 eu,[29] probably indicating an absence of extra solvent structure in the transition state for the exchange reaction and that most of the proton transfer occurs by an intramolecular or "one-encounter" mechanism.[30] Intramolecular proton transfer in acetic acid has been demonstrated to occur through two intervening molecules of water,[31] but the method used would not detect direct transfer not involving solvent.

A second possible explanation for high values of $k_{\mathrm{h}}/k_{\mathrm{ex}}$ can be derived from a possible abnormal affinity of OH^- for acyl carbon as compared to the proton. The magnitude of this enhancement can be seen from an extrapolation of the straight-line relationship correlating the logarithms of the equilibrium constants for the formation of acetate esters from acetic acid with the pK_{a} of the alcohol to include the exchange of the OH moiety of acetic acid, a reaction having an equilibrium constant of 1.[7c,32] This procedure reveals that hydroxide ion as an acyl acceptor is comparable to the conjugate base of an alcohol of p$K_{\mathrm{a}} \sim 17$. It is interesting that extrapolation of the plot of log $k_{\mathrm{h}}/k_{\mathrm{ex}}$ *vs.* pK_{a} of the alcohol to $k_{\mathrm{h}}/k_{\mathrm{ex}} = 1$ (the reaction of OH^- with acetic acid) gives a similarly high value for the pK_{a} of water suggesting that the high affinity is retained in the tetrahedral intermediate. Chemically this might be attributed to hydrogen bonding across the vertices of the intermediate providing additional stability to the tetrahedral adduct. The high value of the association constant of OH^- with an ester cannot be the result of an extraordinary nucleophilic reactivity of OH^- toward acyl carbon since its rate of reaction with *p*-nitrophenyl acetate is particularly low when compared to alkoxide ions of similar basicity.[33] This fact emphasizes the probability that the rate of dissociation of OH^- from a tetrahedral intermediate is considerably less than that predicted from a linear free energy relationship based on proton affinity alone.



The third possibility which can be considered as a limiting case of the first is that no tetrahedral intermediate is formed at all but that the exchange and hydrolysis reactions proceed by parallel independent pathways.[9] The recent unambiguous kinetic demonstrations of intermediates in acyl transfer reactions militate against this last interpretation.[3-5]

The above arguments do not account for the oxygen-18 exchange results obtained for the alkaline hydrolysis of benzamide. The value of $k_{\mathrm{h}}/k_{\mathrm{ex}}$ reported for this compound is 0.53[23] at 40.7°, while the estimated pK_{a} of NH_3 is 35.[34] Structure–reactivity considerations indicate, however, that other factors than hydroxide ion attack are involved in the rate-determining step in the alkaline hydrolysis of amides since ρ values for a number of acyl-activated benzoate esters are near 2.0 at 25° [35] while that calculated for substituted benzamides is 1.36 at 52.8°.[36]

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[27] J. Hine and M. Hine, *J. Amer. Chem. Soc.*, 74, 5266 (1952).

[28] M. L. Bender and R. D. Ginger, *Suomen Kemistilehti, B*, 33, 25 (1960), and this report.

[29] Calculated from the data of ref 23.

[30] M. Eigen, *Discussions Faraday Soc.*, 39, 7, 46 (1965); J. E. Reimann and W. P. Jencks, *J. Amer. Chem. Soc.*, **88**, 3973 (1966).

[31] Z. Luz and S. Meiboom, *ibid.*, **85**, 3923 (1963).

[32] J. Gerstein and W. P. Jencks, *ibid.*, **86**, 4655 (1964).

[33] W. P. Jencks and M. Gilchrist, *ibid.*, **84**, 2910 (1962).

[34] R. P. Bell, "The Proton in Chemistry," Cornell University Press, Ithaca, N. Y., 1959, p 88.

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[36] H. H. Jaffé, *Chem. Rev.*, **53**, 191 (1953).