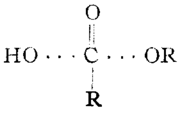
[CONTRIBUTION FROM THE GEORGE HERBERT JONES LABORATORY, UNIVERSITY OF CHICAGO]

Oxygen Exchange as Evidence for the Existence of an Intermediate in Ester Hydrolysis[1]

BY MYRON L. BENDER[2]

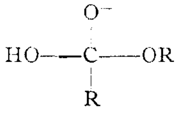
Ethyl benzoate, *i*-propyl benzoate and *t*-butyl benzoate have been labeled in the carbonyl group with O^{18}. The kinetics of the hydrolysis of these esters, and of the oxygen exchange accompanying hydrolysis, have been determined. The fact that oxygen exchange occurs in every case between the carbonyl oxygen of the ester and the solvent during the process of hydrolysis strongly suggests that a true intermediate, in which the carbonyl oxygen participates in a reversible fashion, occurs during the hydrolysis. The most probable structure of this intermediate must include a contribution from the un-ionized hydrate of the ester, $C_6H_5{-}C(OH)_2(OR)$, since the intermediate must assume a symmetrical structure capable of exchange. In 33% dioxane, k_h/k_e for ethyl benzoate is 10.6; for *i*-propyl benzoate it is 3.7; for *t*-butyl benzoate it is 7.6. The similarity of k_h/k_e for these three esters and the virtual identity of k_h/k_e for both the acid and alkaline hydrolysis of ethyl benzoate sugggest a similar intermediate in all cases.

From a multitude of proposals for the hydrolyses of those simple carboxylic esters which occur by means of a bimolecular reaction involving acyl-ion fission, two outstanding sets of mechanisms have been put forth, one by Hammett[3] and another by Day and Ingold.[4] The formulations of both these authors fit the kinetic, optical activity and isotope exchange data available in the literature. Experiments utilizing O^{18} have demonstrated that the rupture of simple carboxylic esters takes place with acyl-ion fission during both alkaline[5] and acid[6] hydrolysis. Hammett proposes, on the basis of this and other evidence, that alkaline hydrolysis involves the addition of hydroxyl ion to the carbonyl carbon, followed or accompanied by the release of the alkoxyl group, and that acid hydrolysis probably involves the addition of water to the carbonyl double bond with the monoester of the ortho acid as an intermediate. Day and Ingold illustrate alkaline hydrolysis with two mechanisms, one involving an addition intermediate, the other a transition state. They illustrate acid hydrolysis with a mechanism involving a transition state. In discussing the base-catalyzed mechanism, Day and Ingold state that I and II "differ only in electron distribution, and are clearly to be regarded as imperfect representations of a mesomeric system having an intermediate electronic arrangement." The two sets of mechanisms leave unanswered the question of the nature of the intermediate substances in these reactions. Day and Ingold state "the question may one day arise, whether the most condensed intermediate state occurs at the energy maximum or before or after it; but such distinctions are not in the present program."



$$\mathrm{HO\cdots\underset{\underset{R}{|}}{\overset{\overset{O}{\|}}{C}}\cdots OR}$$

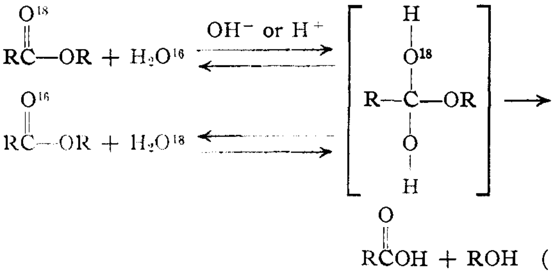
I



$$\mathrm{HO{-}\!\!{-}\underset{\underset{R}{|}}{\overset{\overset{O^-}{|}}{C}}{-}\!\!{-}OR}$$

II

The present research was designed to furnish evidence with which to distinguish between an intermediate and a transition state in the hydrolysis reaction. The experimental approach was based on an hypothesis made by Roberts and Urey[7] that esterification and oxygen exchange of acids and hydrolysis of esters occur through the same mechanism. In the present research a study was made of the base- and acid-catalyzed oxygen exchange of ethyl benzoate, *i*-propyl benzoate and *t*-butyl benzoate with water; that is, the oxygen exchange which occurred during the alkaline and acid hydrolysis of these esters. It should be noted that amyl acetate has been reported to give no exchange with water containing excess O^{18}[8]; however, the present research deals with oxygen exchange during hydrolysis as opposed to an exchange without chemical reaction. The esters, labeled with excess O^{18} in the carbonyl group, were subjected to experiments outlined by the schematic equation (1)



$$\begin{array}{l}\mathrm{R\overset{\overset{O^{18}}{\|}}{C}{-}OR + H_2O^{16}} \\ \mathrm{R\overset{\overset{O^{16}}{\|}}{C}{-}OR + H_2O^{18}}\end{array} \begin{array}{c}\xrightleftharpoons{\text{OH}^- \text{ or H}^+} \\ \rightleftharpoons\end{array} \left[\mathrm{R{-}\underset{\underset{\underset{H}{|}}{\overset{|}{O}}}{\overset{\overset{\overset{H}{|}}{\underset{|}{O^{18}}}}{C}}{-}OR}\right] \longrightarrow \mathrm{R\overset{\overset{O}{\|}}{C}OH + ROH} \quad (1)$$

The presence of a true intermediate in the hydrolysis will be detected, then, by the fact that under *partial* hydrolysis the *remaining ester* will be expected to contain less O^{18} in the carbonyl group than it had originally. On the other hand, no oxygen exchange will be expected to occur if a straight substitution reaction were operative for then the carbonyl oxygen would not be a participant in any reversible step. This formulation assumes that there is no reverse (esterification) reaction; this is certainly true during alkaline hydrolysis; while acid hydrolysis is a reversible reaction, the initial concentrations of reactants limited the reverse reaction to less than 0.01%.

Experimental

Materials.—Water containing *ca.* 1.5 atom % of O^{18} was supplied by the Stuart Oxygen Co., San Francisco, Calif., through the Atomic Energy Commission. Water used as solvent in the exchange experiments was distilled water which was redistilled from potassium permanganate in a tin still. Dioxane (Carbide and Carbon Co.) was purified by

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[1] Presented at the Chicago Meeting of the American Chemical Society, September, 1950.

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[3] Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., New York, N. Y., 1940, pp. 355–357.

[4] Day and Ingold, *Trans. Faraday Soc.*, **37**, 686 (1941).

[5] Polanyi and Szabo, *ibid.*, **30**, 508 (1934).

[6] Datta, Day and Ingold, *J. Chem. Soc.*, 838 (1939).

[7] Roberts and Urey, THIS JOURNAL, **61**, 2584 (1939).

[8] Cohn and Urey, *ibid.*, **60**, 679 (1938).

the method described by Fieser.[9] Methylene dichloride was a Matheson Co. product. The sodium hydroxide solutions used as catalyst and as titrant were standardized against standard potassium acid phthalate using phenolphthalein as indicator. Hydrochloric acid solution was standardized against a standard sodium hydroxide solution using phenolphthalein as indicator. Perchloric acid used as acid catalyst was a Baker C.P. product. Ethyl benzoate (normal isotopic composition) was an Eastman Kodak Co. White Label product, n^{20}D 1.5055. *i*-Propyl benzoate (normal isotopic composition) was an Eastman Kodak Co. White Label product, n^{20}D 1.4948. *t*-Butyl benzoate (normal isotopic composition) was prepared from *t*-butyl alcohol (Eastman Kodak Co. White Label) and benzoyl chloride (Mallinckrodt reagent) in pyridine (Merck and Co., Inc. reagent) as solvent according to the directions of Norris and Rigby[10]; b.p. 67–69° (1 mm.); n^{25}D 1.4894.

Ethyl Benzoate (Labeled with O^{18} in the Carbonyl Group).—Benziminoethyl ether hydrochloride was prepared from benzonitrile (redistilled Eastman Kodak Co. White Label), absolute ethanol (dried by means of the diethyl phthalate method[9]), and gaseous hydrogen chloride according to the method of Pinner[11]; m.p. 117–120° dec.

Benziminoethyl ether hydrochloride (46 g., 0.248 mole) was dissolved in a mixture of 46.2 ml. of doubly distilled water and 3.8 ml. of water containing *ca.* 1.5 atom % of O^{18}. The mixture was heated on the steam-bath for one hour with occasional shaking. The mixture was cooled; the organic layer was separated, washed with water, dried with sodium sulfate, and distilled through a column packed with glass helices. Thirty grams of labeled ethyl benzoate was collected; 81%; b.p. 90° (14 mm.); n^{20}D 1.5053.

Benzoic Acid (Labeled with O^{18}).—Benzoic acid (Eastman Kodak Co. White Label) (4 g.) was dissolved in a mixture of 27.6 ml. of doubly distilled water and 22.4 ml. of water containing 1.5 atom % of O^{18}. The solution was made 0.1 *N* in hydrochloric acid (Baker C.P.) and refluxed for 20 hours. The solution was cooled in ice-water and the labeled benzoic acid was filtered and dried in vacuum. Essentially a quantitative recovery was made. The heavy-water filtrate was used over in four subsequent similar runs. This procedure follows that of Roberts and Urey.[12]

***i*-Propyl Benzoate (Labeled with O^{18} in the Carbonyl Group).**—Labeled benzoic acid as prepared above (22 g., 0.18 mole) and thionyl chloride (27 g., 0.226 mole) were mixed, allowed to stand at room temperature for two hours, and then refluxed for two hours. The excess thionyl chloride and hydrogen chloride were removed in vacuum. Isopropyl alcohol (b.p. 82.3°) (20 g.) was added and the solution was refluxed for 12 hours. The reaction product was washed with a 5% potassium carbonate solution, washed with water, dried with sodium sulfate and distilled through a tantalum wire column. Twenty-five grams of labeled *i*-propyl benzoate was obtained; 85%; b.p. 71–72° (5 mm.); n^{20}D 1.4949.

***t*-Butyl Benzoate (Labeled with O^{18} in the Carbonyl Group).**—Labeled benzoyl chloride, as prepared above from labeled benzoic acid (22 g., 0.18 mole), was mixed with *t*-butyl alcohol (24.7 ml.) and pyridine (31.3 ml.); the mixture was left overnight. The reaction product was extracted with water twice, 0.1 *N* hydrochloric acid four times, and finally with water twice. The crude ester was dried over sodium sulfate and distilled through a tantalum wire column. Twenty-two grams of labeled *t*-butyl benzoate was obtained; 66%; b.p. 67–68° (1 mm.); n^{25}D 1.4890. This preparation follows the procedure of Norris and Rigby.[10]

Kinetics of Hydrolysis.—Although the rates of hydrolysis of the esters, ethyl benzoate, *i*-propyl benzoate and *t*-butyl benzoate, have been determined in many solvents, it was desirable to find the specific rate constants for their hydrolysis in pure water and in 33% dioxane–water. The hydrolyses were followed at three temperatures according to the ester and the solvent used. A water thermostat was used at 25.12 ± 0.02°. An oil thermostat was used at 62.5 ± 0.1°. A refluxing aqueous solution was used as a crude thermostat for one run; it was assumed to be at 99 ± 1°. To check the purity of the labeled esters, the hydrolyses of the labeled and unlabeled esters were run in parallel experiments.

The hydrolyses were carried out by dissolution of the ester in the solvent (by mechanical or manual shaking), equilibration of the solution in a thermostat, and addition of the appropriate reagent. The rates were followed by removal of suitable aliquots and titration with standard sodium hydroxide or hydrochloric acid, using phenolphthalein as indicator. In the alkaline hydrolyses, the two components, ester and hydroxide ion, were initially present in equal concentration; a plot of $1/(a - x)$ (a = initial concentration, x = concentration reacted) *versus* t (t = time) gave a straight line in every case with a slope equal to the second-order rate constant. The concentrations of both ester and hydroxide ion were in the range of 0.001–0.007 *M*. The pseudo first-order rate constant in the case of acid hydrolysis was determined from the slope of the plot of log. $(a - x)$ *versus* t. A blank experiment showed that no isobutylene was formed in the hydrolysis of *t*-butyl benzoate. The kinetic data are shown in Fig. 1.

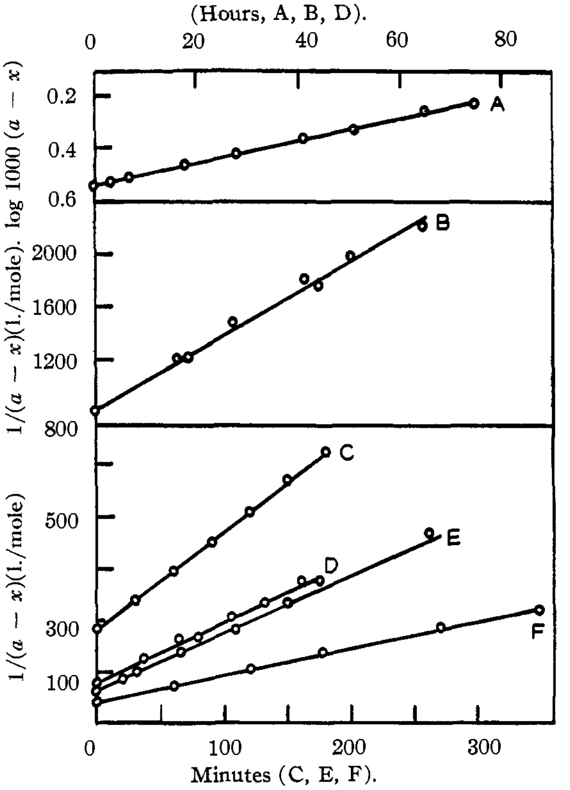

Fig. 1.—Kinetics of hydrolysis:

	Water			33% Dioxane	
A	Ethyl benzoate	H^+	D	*t*-Butyl benzoate	OH^-
B	*i*-Propyl benzoate	OH^-	E	*i*-Propyl benzoate	OH^-
C	Ethyl benzoate	OH^-	F	Ethyl benzoate	OH^-

Oxygen Exchange Experiments.—Samples of the labeled esters (usually 0.5 to 1.0 g.) were hydrolyzed in doubly distilled water or 33% dioxane–water under conditions similar to those used in the determination of the rates of hydrolysis. After appropriate amounts of time which corresponded to conversions ranging from 0–80% hydrolysis, the remaining ester was isolated and analyzed as described in a later section. The isolation of the esters from water was accomplished by stopping the reaction by the addition of sodium bicarbonate and cooling, followed by extraction with five 15-ml. portions of methylene dichloride, drying with sodium sulfate and removal of the methylene dichloride on a hot-plate. The isolation of the esters from 33% dioxane–water was accomplished by the addition of sodium bicarbonate and cooling, followed by extraction with two 15-ml. portions of methylene dichloride and removal of the solvents (dioxane and methylene dichloride) at 30–40° in vacuum. This procedure was needed especially for *t*-butyl

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[9] Fieser, "Experiments in Organic Chemistry," D. C. Heath and Co., New York, N. Y., 1941.

[10] Norris and Rigby, This Journal, **54**, 2098 (1932).

[11] Pinner, *Ber.*, **16**, 1654 (1883).

[12] Roberts and Urey, This Journal, **61**, 2580 (1939).

benzoate which is easily decomposed at elevated temperatures. All the isolation procedures, when tested with blanks, gave recoveries of 95% or better.

Analysis of O^{18} Content of the Esters.—The various esters were converted to carbon dioxide in two steps in essentially quantitative yield. These carbon dioxide samples contained the two oxygen atoms of the original esters and thus the measurement of the O^{18} content of the carbon dioxide samples was a direct measure of the O^{18} content of the esters. The first step in the conversion to carbon dioxide was the thermal decomposition of the ester into benzoic acid and an olefin. According to Bilger and Hibbert,[13] the decomposition of ethyl benzoate into benzoic acid and ethylene is virtually quantitative. By passing 0.1-g. samples of ethyl benzoate through a 10-inch tube (Pyrex 172, 12 mm. i.d.) at approximately 600° using a stream of prepurified nitrogen as carrier (flow = 40 cc./min.), it was possible reproducibly to isolate benzoic acid (m.p. 120–121°) in 97% yield. The decomposition of *i*-propyl benzoate and of *t*-butyl benzoate can be effected at successively lower temperatures but for convenience all decompositions were carried out as described above. The benzoic acid samples obtained by decomposition of the latter esters were equally as good as those from ethyl benzoate with respect to yield and to melting point.

The second step in the conversion was the decarboxylation of benzoic acid to produce the desired carbon dioxide and benzene. The procedure and apparatus for this reaction were fashioned from the directions of Roberts and Urey.[12] The copper used for the decarboxylation was in the form of fine shot (Mallinckrodt analytical reagent) and was activated by treatment with gaseous ammonia at 500°. The decarboxylations were run at 500 ± 20° usually with 20-mg. samples of benzoic acid. The decarboxylation furnace was made of ordinary Pyrex tubing (12 in. × 20 mm. i.d.); a thermocouple well was inserted in the middle of the furnace. Prepurified nitrogen, further purified by passage through a copper tower at 500° and through a drying tube containing drierite and finally phosphorus pentoxide, was used as carrier for the benzoic acid at a flow rate of 15 cc./min. The yield of carbon dioxide was essentially quantitative.

The O^{18} content of the carbon dioxide samples was measured by means of a modified Nier-type isotope-ratio mass spectrometer in which the masses 46 and 44 are collected simultaneously. This instrument is described by McKinney, McCrea, Epstein, Allen and Urey.[14]

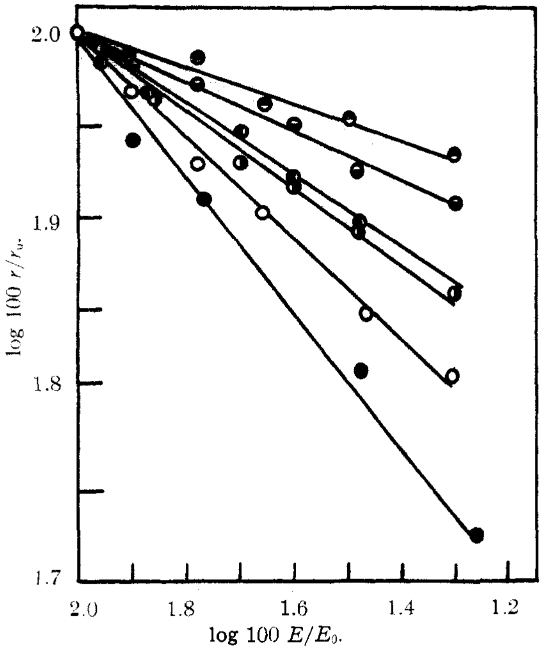

Fig. 2.—Oxygen exchange *vs.* hydrolysis:

33% Dioxane			H_2O		
●	Ethyl benzoate	OH^-	◑	Ethyl benzoate	OH^-
○	*i*-Propyl benzoate	OH^-	◐	Ethyl benzoate	H^+
◒	*t*-Butyl benzoate	OH^-	●	*i*-Propyl benzoate	OH^-

Results and Calculations

The rates of the alkaline hydrolysis of both labeled and unlabeled ethyl benzoate, *i*-propyl benzoate and *t*-butyl benzoate have been determined in water and in 33% dioxane–water. The rate of acid hydrolysis of ethyl benzoate in water has also been determined. The rate constants for these reactions calculated from the experimental data given in Fig. 1 are presented in Table I.

TABLE I

SPECIFIC RATE CONSTANTS OF HYDROLYSIS

Benzoate	Water k_2 (l./mole sec.)	Temp., °C.	33% Dioxane–Water k_2 (l./mole sec.)	Temp., °C.
	Alkaline hydrolysis			
Ethyl	3.0×10^{-2}	25.12	8.7×10^{-3}	25.12
i-Propyl	0.62×10^{-2}	25.12	1.8×10^{-2}	62.5
t-Butyl	[a]		1.3×10^{-3}	62.5
	Acid hydrolysis			
	k_1 ($H^+ = 1$ *M*) (sec.$^{-1}$)			
Ethyl	3.9×10^{-4}	99		

[a] This experiment and the corresponding exchange experiment were not performed because of the sparing solubility of *t*-butyl benzoate in water.

Exchange experiments were performed with ethyl benzoate during both acid and alkaline hydrolysis and with *i*-propyl benzoate and *t*-butyl benzoate during alkaline hydrolysis. In every case exchange took place between the solvent and the ester during the course of the hydrolysis. The rates of oxygen exchange of the various esters were determined from the raw isotope exchange data by means of the formulation

E = total ester

E' = ester containing excess O^{18} (over the normal composition)

$$E \xrightarrow{k_h} \text{acid} + \text{alcohol}$$

$$E' \xrightarrow{k_e} \text{unlabeled ester}$$

$$-\mathrm{d}(E')/\mathrm{d}t = k_h(E') + k_e(E')$$

$$-\mathrm{d}(E)/\mathrm{d}t = k_h(E)$$

$$\frac{\mathrm{d}(E')}{\mathrm{d}(E)} = \frac{(k_h + k_e)(E')}{k_h(E)}$$

$$k_h \ln \frac{(E')}{(E'_0)} = (k_e + k_h) \ln \frac{(E)}{(E_0)}$$

$$r = E'/E = \text{excess atom fraction } O^{18}$$

$$k_h \ln r/r_0 = k_e \ln (E)/(E_0)$$

This result requires that the plot of the logarithm of the excess atom fraction of O^{18} *versus* the logarithm of the ester concentration give a straight line with a slope equal to the ratio of the exchange rate constant to the hydrolytic rate constant. Plots such as are described above are shown in Fig. 2 where both r/r_0, the excess atom fraction of O^{18}, and E/E_0 are expressed in terms of per cent. for convenience. The ratio r/r_0 was calculated as Enrichment/Enrichment$_0$ where

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(13) Bilger and Hibbert, THIS JOURNAL, **58**, 823 (1936).

(14) McKinney, McCrea, Epstein, Allen and Urey, *Rev. Sci. Inst.*, in press.

$$\text{Enrichment} = \frac{(\text{Mass } 46/\text{Mass } 44)_x - (\text{Mass } 46/\text{Mass } 44)_{\text{standard}}}{(\text{Mass } 46/\text{Mass } 44)_{\text{standard}}}$$

In every case a reasonably straight line was obtained. The ratios of $k_{\text{hydrol.}}$ to $k_{\text{exch.}}$ calculated from the slopes of these lines are given in Table II.

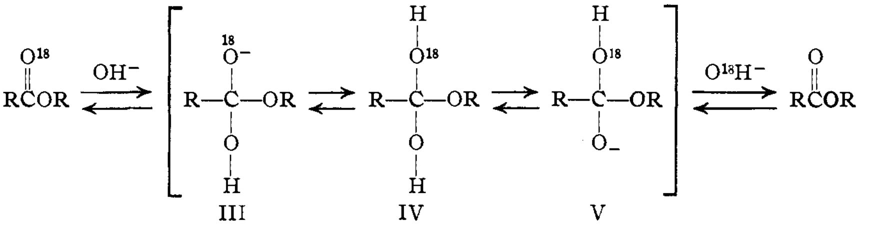
TABLE II

RATIOS OF $k_{\text{hydrol.}}$ TO $k_{\text{exch.}}$

Benzoate	Water	33% Dioxane–Water
	Alkaline hydrolysis	
Ethyl	4.8	10.6
i-Propyl	2.7	3.7
t-Butyl		7.6
	Acid hydrolysis	
Ethyl	5.2	

Conclusions

Oxygen exchange has been shown to occur in six cases between the carbonyl oxygen of the ester and the solvent during the process of hydrolysis. The rates of exchange and of hydrolysis are similar (in all cases within a factor of 10), suggesting a similar mechanism for these two processes. A reasonable explanation for these facts is that a true intermediate, in which the carbonyl oxygen of the ester participates in a reversible fashion, occurs during hydrolysis. The exchange indicates further that the un-ionized ester hydrate, IV, must be a contributor to the structure of the intermediate, for only then can the intermediate assume a symmetrical structure capable of exchange. Equation (2) illustrates the proposed exchange process during alkaline hydrolysis.



$$R\overset{O^{18}}{\overset{\|}{C}}OR \overset{OH^-}{\rightleftharpoons} \left[\underset{\text{III}}{R-\overset{{}^{18}O^-}{\underset{OH}{C}}-OR} \rightleftharpoons \underset{\text{IV}}{R-\overset{HO^{18}}{\underset{OH}{C}}-OR} \rightleftharpoons \underset{\text{V}}{R-\overset{HO^{18}}{\underset{O_-}{C}}-OR} \right] \overset{O^{18}H^-}{\rightleftharpoons} R\overset{O}{\overset{\|}{C}}OR \quad (2)$$

The rate of exchange will then be a measure of the re-formation of the ester from the intermediate. More precisely the ratio of k_h/k_e will be approximately two times the ratio of the rate of breakage of the C–OR bond to that of the C–OH bond since IV contains two C–OH bonds, only one of which contains an O^{18}. (The factor of two is only approximate because of the effect of the small difference in zero-point energies in the bonds involving O^{16} and O^{18} both on formation of the ions, III and V, and on their breakdown.)

The constancy of k_h/k_e for ethyl benzoate, *i*-propyl benzoate, and *t*-butyl benzoate (within a factor of four) suggests a similar intermediate in all cases. The fact that k_h/k_e is approximately the same for both the acid and alkaline hydrolysis of ethyl benzoate (although the respective rates of hydrolysis extrapolated to the same temperature differ by more than a factor of 10^4) indicates that in both these reactions a similar intermediate is formed, the most likely possibility in both cases being the un-ionized hydrate of the ester.

The considerable decrease in the ratio of k_h/k_e in going from 33% dioxane to water may be explained in the following fashion. Assuming that the ratio of k_h/k_e is determined by the relative ease of formation of the alkoxyl anion to that of the hydroxyl ion from the intermediate, the increased solvation of the hydroxyl ion relative to that of an alkoxyl ion in a solvent of higher dielectric constant such as water will increase the exchange and thus decrease the ratio of k_h/k_e. No ready explanation in terms of electronic theory can be offered for the changes that occur in the ratio of k_h/k_e with changes in the alkoxyl group. These changes may be linked to the acid strengths of the corresponding alcohols but no clear correlation can be noted.

Although this research has been concerned with the hydrolytic process, the reverse reaction of esterification could be considered to proceed through a similar intermediate without an excessive amount of extrapolation.

Acknowledgment.—The author wishes to express his appreciation to Dr. F. H. Westheimer for many stimulating discussions in all phases of this research. Thanks are also due to Dr. Jack Hine of the Georgia Institute of Technology for discussion at the inception of this problem and to Dr. H. C. Urey and Dr. Samuel Epstein through whose courtesy the mass spectrometer was made available. A grant from the Atomic Energy Commission made this work possible.

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