

THE ISOTOPE EFFECT IN THE ALKALINE HYDROLYSIS
OF METHYL p-METHYL-t-BENZOATE

By

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INTRODUCTION

Substitution of various groups on the benzene ring has a pronounced effect on the rates at which the ring-substituted esters of benzoic acid will hydrolyse in aqueous, alkaline solution (10). The rate of the reaction is dependent on the concentration of both the ester and of the base. In general, the rate of hydrolysis is increased by substitution of electronegative groups on the ring.

The relative rates of hydrolysis of methyl p-methyl-t-benzoate and its protium analog should be a function of the difference in the inductive and resonance effects of tritium and protium.

The purpose of the research presented in this thesis was to measure the relative rates of alkaline hydrolysis of methyl p-methyl-t-benzoate and the protium analog.

HISTORICAL

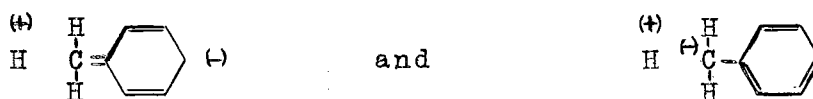
The Inductive Effect

The fact that some atoms and groups of atoms have a stronger attraction for electrons than others has been recognized for a long time. The inductive effect results from the displacement of the electron pair of a bond toward one of the atoms of the bond, without any actual transfer from one atom to another. The inductive effect therefore, depends only on the attraction of an atomic nucleus for its valency electrons and the resulting screening of the nucleus. Since the carbon atom demonstrates a stronger attraction for electrons than the hydrogen atom, in a group such as the methyl group the electron density is displaced toward the carbon atom and results in a displacement toward an adjacent atom or group. For example, the following aliphatic hydrocarbon groups are listed in order of their increasing electron-releasing abilities, CH_3- , CH_3CH_2- , $(\text{CH}_3)_2\text{CH}-$, $(\text{CH}_3)_3\text{C}-$.

The Baker-Nathan Effect

In studying the rates of reaction of p-alkylbenzyl bromides with pyridine, Baker and Nathan (2) found relative reaction rates that would not be expected on the basis of inductive effects of the substituent groups. Berliner and

Berliner (4) found that the relative rates of bromination of several monoalkylbenzenes were also the reverse of those expected on the basis of induction alone. A large number of other cases of this effect are known and several attempts have been made to explain this phenomenon (5) (15). So far the most widely accepted explanation has been that of hyperconjugation, which can be defined as the contribution of such resonance structures as

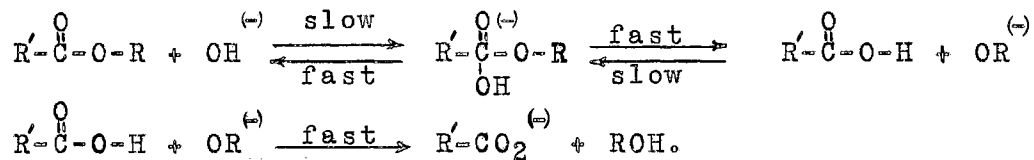


to the overall resonance structure of the molecule.

Recently McKreevoy and Eyring (13) have suggested that the Baker-Nathan effect might be satisfactorily explained by a small but non-zero resonance integral for the hydrogen 1s orbital with an orbital one atom removed from it.

Rates of Hydrolysis of p-Alkylbenzoate Esters.

The mechanism for the alkaline hydrolysis of esters is believed to be:



The rate-determining step is the attack of the hydroxide ion on the carboxyl carbon.

Several studies of the effects of various para-substituted groups on the rate of alkaline hydrolysis of benzoate esters have been made. Table I indicates that strong electron-withdrawing groups speed the reaction (10).

TABLE I

RELATIVE RATES OF SECOND-ORDER ALKALINE HYDROLYSISp-XC₆H₄CO₂C₂H₅ in 85% aqueous EtOH at 25°

<u>X</u>	<u>Relative Rate</u>	<u>X</u>	<u>Relative Rate</u>
-NH ₂	0.023	-Cl	4.31
-OCH ₃	0.209	-Br	5.25
-CH ₃	0.456	-I	5.05
-H	1.000	-NO ₂	110.00
-F	2.03		

From Table I it can be seen that the rate for the p-methyl substituted compound is lower than the reaction rate of the unsubstituted ethyl benzoate. The contribution of resonance effects alone is not readily apparent.

Another study of this same type of reaction has been made by Herbst and Jacox (7) with various ethyl p-alkylbenzoates in 87.8 weight per cent ethanol at 30°. They found the order of rate constants to follow the sequence which would be expected on the basis of inductive effects alone.

Another study by Berliner and others (3) using the same compounds at 25° showed that the inductive order was followed in 56 weight per cent acetone, but that the reverse order was followed in 85 weight per cent ethanol.

Isotope Effects of Hydrogen

Due to the large differences in the relative masses of the isotopes of hydrogen, this element gives larger isotope effects than any other element. If the bond force constants are essentially the same for each isotope, then heavier isotopes will have lower vibrational frequencies. According to

Hooke's Law, for most bonds the frequencies would vary inversely as the square root of the reduced masses of each pair of atoms.

At moderate temperatures nearly all of the carbon-hydrogen bonds are at their zero-point vibrational energy level, in which

$$E = \frac{1}{2} h \nu$$

where E represents vibrational energy, h is Planck's constant, and ν is the vibrational frequency of the bond. Therefore, the heavier isotopes have the lower vibrational energy.

The potential energy curves are essentially the same for the bond between a particular atom and the isotopes of another atom. Consequently, the bonds of the heavier isotopes are more stable and, in a reaction involving isotopic bond cleavage, a higher activation energy is generally required (Figure I).

The difference in reaction rates when the isotopic bond is broken in the rate-determining step has been experimentally demonstrated many times. In some cases the rate has been found to be as much as ten times as fast for the protium compound as compared to the tritium compound.

In the case of the hydrogen isotopes it is not always necessary that the isotopic bond be broken in order to have an isotope effect. This has been demonstrated by Lewis and Coppinger in studying the rates of acetolysis of variously deuterated methyl-p-tolylcarbonyl chlorides (12) (Table II). The mechanism of this reaction is believed to be unimolecular and to have a carbonium ion intermediate.

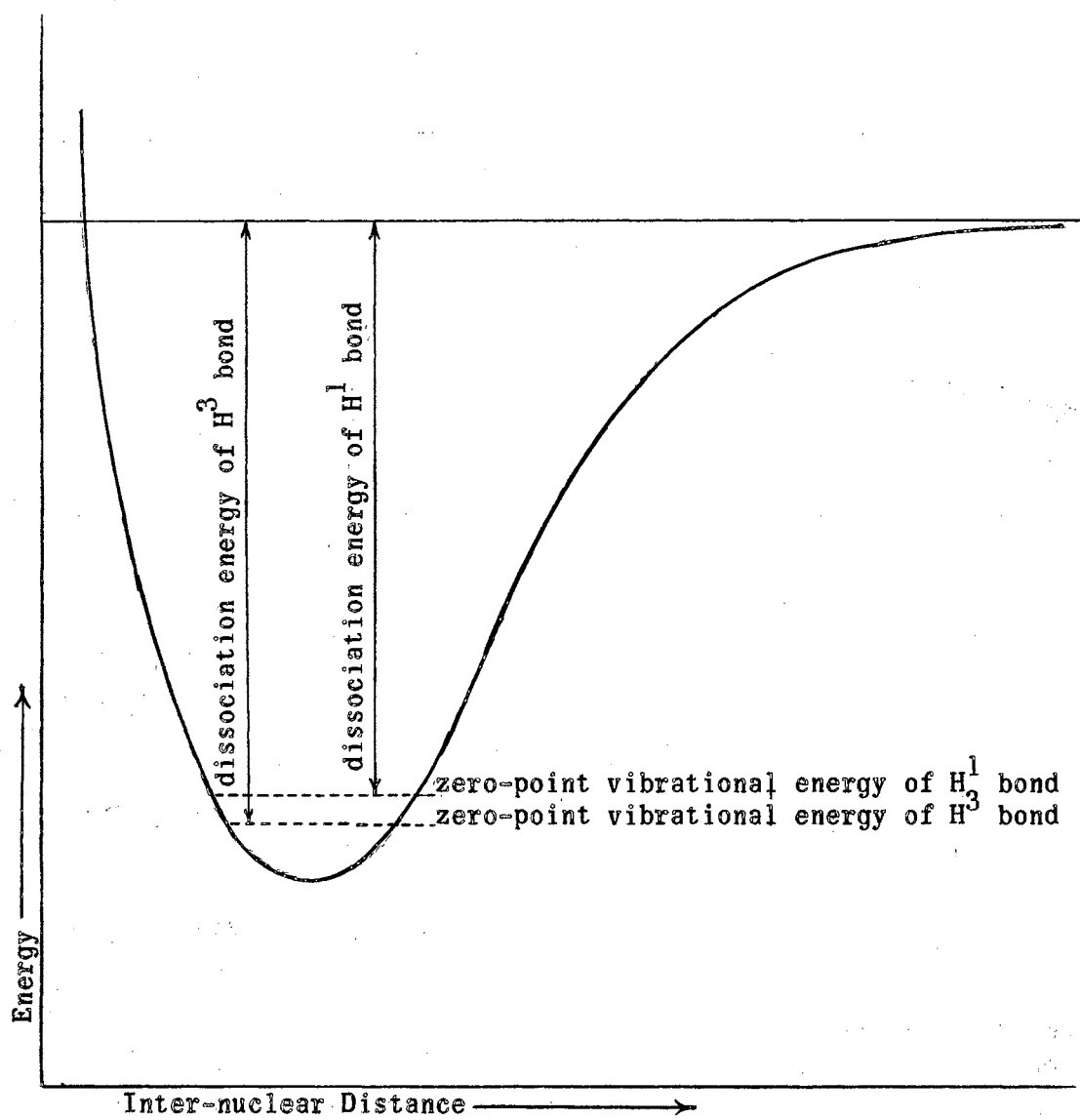


Figure 1: Potential Energy Curve

TABLE II

RATES OF ACETOLYSIS OF METHYL p-TOLYLCARBINYL CHLORIDE

Compound	Deuterium in Methyl Group	Temperature	k x 10 ⁴
	%	°C.	sec. ⁻¹
CH ₃ C ₆ H ₄ CHClCH ₃	0	50.25	1.14
CH ₃ C ₆ H ₄ CHClCH ₃	0	65.30	6.02
CD ₃ C ₆ H ₄ CHClCH ₃	67	50.25	1.04
CD ₃ C ₆ H ₄ CHClCH ₃	67	65.30	5.61

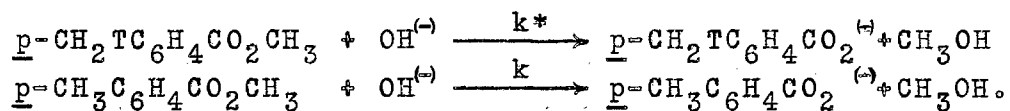
Since the formation of a carbonium ion in the rate-determining step would be facilitated by electron release from the methyl group, it appears that a heavily deuterated methyl group is a weaker electron donor than the protium analog.

INTRODUCTION TO EXPERIMENTAL WORK

General Procedure

The intermolecular isotope effect is the ratio of reaction rates for molecules which are identical except for the presence of different isotopes of the same atom.

The purpose of this investigation was to measure the intermolecular isotope effect in the following reaction:



The method used in determining the relative rates was to hydrolyze samples of the ester containing tracer amounts of the labeled ester to various known stages of completion. The tritium contents of the acids from the partial hydrolysis and from the complete hydrolysis were determined by measuring the radioactivities of the acids produced. The ratio of the rate constants was determined by application of the equation of Stevens and Attree (17),

$$\frac{k^*}{k} = \frac{\log (1 - r f)}{\log (1 - f)}$$

where f is the fraction reacted and r is the ratio of the specific activity of acid produced to that of the original ester.

Assay Method

A sample of approximately 5 mg. was weighed and placed in a break-off tube with 1 g. of zinc, 100 mg. of nickelic oxide and a small sealed ampule containing about 5 mg. of water. The tube was then evacuated, sealed and placed in a furnace at 625° for three hours (16). At the end of this period the tritium should be in a gaseous form as either hydrogen or methane. A known fraction of this gas was then transferred to an ionization chamber. Additional methane was added to the ionization chamber to a pressure of one atmosphere and the activity of the gas in the chamber was determined with a vibrating reed electrometer.

EXPERIMENTAL WORK

Preparation of Tritiated Water

The tritiated water which was used in this work was prepared by passing a mixture of hydrogen and 100 millicuries of tritium gas over hot copper oxide by means of a Toepler pump, according to the method of Hodnett, Feldman and Flynn (9). The tritiated water obtained weighed 10.339 g. and had an activity of 4.92 $\mu\text{c./mg.}$

Attempted Preparation of p-Methyl-t-benzoic Acid

An attempt was made to tritiate p-toluic acid by the substitution of a hydrogen atom of the methyl group with a tritium atom of tritiated water. This was done by placing 0.119 g. of p-toluic acid and 1 ml. of tritiated water in a sealed ampule and placing the ampule in a steam bath for 50 hours. The ampule was opened and the water distilled off on a vacuum line. Then 0.5 ml. of ethanol and 1 ml. of distilled water was added to the remaining p-toluic acid and the mixture was allowed to stand for 20 minutes at room temperature. The solvent was then distilled off on the vacuum line and the activity of the acid was found to be 0.006 $\mu\text{c./mg.}$

It was felt that since the acid had not completely dissolved in the alcohol-water mixture, some of this activity must still be on the carboxyl group. Therefore, 0.05 g. of

the acid was dissolved in 2.0 ml. of 1 N sodium hydroxide solution and precipitated by adding hydrochloric acid to a total volume of about 6 ml. The acid was then separated and washed twice with 5 ml. of distilled water. The acid, when dried, was found to have an activity of 0.001 $\mu\text{c.}/\text{mg.}$, indicating that little, if any, substitution of tritium for protium had occurred in the acid.

Preparation of α -t-Toluene

Two and one-half grams of dry magnesium turnings was placed in a dry 100-ml. three-neck flask fitted with a condenser, an air-tight stirring apparatus and a closed separatory funnel. Five milliliters of anhydrous ether and a few drops of benzyl chloride were added and the mixture allowed to stand until the reaction started. Then the remainder of 10 ml. of benzyl chloride in 30 ml. of anhydrous ether was added to the flask drop-wise.

When the reaction was complete, 1 ml. of tritiated water (4.92 $\text{mc.}/\text{g.}$) was placed in the funnel and admitted to the flask drop-wise with stirring. Ordinary water was then added until the hydrolysis of the Grignard reagent was complete. The ether layer was separated and the ether distilled off. The toluene was distilled off on a vacuum line with approximately seventy percent weight yield and an activity yield of nine percent of the activity of the water which was used. The low activity yield was believed to be due to the loss of tritiated water in the salt crystals

which formed during the hydrolysis.

A better procedure would have been to dissolve the tritiated water in ether before adding it to the flask as was done by Melander (14).

Preparation of p-Methyl-t-acetophenone

Six and one-half milliliters (0.06 moles) of toluene and 12.24 g. (0.09 moles) of anhydrous aluminum chloride with 30 ml. of carbon disulfide were placed in a three-neck flask, fitted with a stirrer, condenser and stoppered separatory funnel. Then 5.35 ml. (0.075 moles) of acetyl chloride was placed in the separatory funnel and added to the reaction flask drop-wise. After all the acetyl chloride had been added, the flask was heated gently until hydrogen chloride was no longer evolved.

After the reaction was complete, the contents of the flask were poured over cracked ice and dilute hydrochloric acid in a separatory funnel. The water layer was removed and the organic layer was washed with 10 ml. of 10 per cent sodium hydroxide solution. The carbon disulfide was then distilled off and 10 ml. of water was distilled through the residue to remove unreacted toluene, leaving about 5 ml. of straw-colored liquid, which was presumed to be p-methyl-t-acetophenone.

Preparation of p-Methyl-t-benzoic Acid

The p-methyl-t-acetophenone from the preparation previously described was dissolved in 30 ml. of methanol and

placed in a 200-ml. three-neck flask fitted with a stirrer, a condenser and a tube for bubbling chlorine gas through the system. The reaction was carried out in a dark room. Sixty milliliters of 20 per cent sodium hydroxide solution was added and chlorine was bubbled into the solution. After chlorine had bubbled in for 15 minutes, an additional 30 ml. of sodium hydroxide solution was added and the bubbling of the chlorine gas continued. Intermittently a small sample was removed from the reaction mixture and dropped into a small amount of potassium iodide solution. When free iodine was detected, the addition of chlorine gas was terminated and 0.5 ml. of acetone was added to react with the excess hypochlorite. The alcohol and chloroform were distilled off and the acid was precipitated by the addition of hydrochloric acid. After separation and drying the acid weighed approximately 4.5 g., melted at 175-177°, literature 179.6° (1), and had an activity of 8 $\mu\text{c.}/\text{mmole}$.

Preparation of Methyl p-Methyl-t-benzoate

The acid from the above oxidation was dissolved in a mixture of 30 ml. of absolute methanol and 0.5 ml. of concentrated sulfuric acid, and the solution placed in a 100-ml. distilling flask fitted with a reflux condenser. After the mixture was refluxed for eight hours, the excess alcohol was distilled off and the ester was separated by adding 20 ml. of distilled water. The water was poured off and the oil was washed with 10 ml. of 5 per cent sodium hydroxide

solution. The oil was separated from the sodium hydroxide solution and again washed with 10 ml. of distilled water.

The oil was allowed to crystallize at room temperature. After four days the crystals were separated from the mother liquor, dissolved in methanol and separated again with distilled water. The oil was again allowed to crystallize at room temperature and the crystals were removed and washed with water. The yield was 3.3 g. of white transparent crystals which melted at 32-33°, literature 33.2° (11).

Hydrolysis of the Ester

Run 1

The tritiated ester was combined with 0.75 g. of ordinary methyl p-toluate which had been prepared in the same way. The two solids were melted together and allowed to crystallize. After the crystals were washed, dried, and weighed, 3.8605 g. was placed in a volumetric flask and dissolved in absolute methanol to a total volume of 25 ml. Then for the 5 per cent hydrolysis, 12.95 ml. of this solution (containing 0.0133 moles of acid) and 5 ml. more of absolute methanol were placed in a flask. Two drops of phenolphthalein solution and 3.635 ml. of 0.183 N sodium hydroxide solution were added and the flask was placed in a water bath at 25° until the phenolphthalein color had faded. At this time the flask was removed from the water bath, the contents frozen, and the alcohol distilled off on the vacuum line. The ester was then separated from the

aqueous phase by extracting twice with 5 ml. portions of ether, and the acid precipitated by the addition of dilute hydrochloric acid. The acid was then separated and the remaining water distilled off on the vacuum line. The dry solid was then vacuum-sublimed over a period of approximately one hour.

The 100 per cent reaction was carried out by adding approximately three times the required amount of sodium hydroxide solution. The reaction mixture stood at room temperature for several days and was then refluxed for two days. At this point the alcohol was distilled off and the sample treated as the 5 per cent sample had been.

A second sublimation was made on each sample and at the end of this sublimation a white, solid residue was found which was very water-soluble and assumed to be sodium chloride crystals which had been carried up during the first sublimation. Radioassays were made on the acids and then a third sublimation was made on the remaining acids and they were assayed again. The results are given in Table III.

Because the activity was increased by further sublimation and because considerable time had elapsed between several of the determinations and it was feared that the absolute reading of the electrometer might have varied over this period of time, it was decided that these data would not be used. Insufficient acid was left for further sublimation, therefore another set of hydrolyses was carried out with some changes made in the purification procedure and technique.

Runs 2 and 3

The unused ester was reclaimed and a small amount of dead ester was added to it. The mixture was melted and washed twice with 0.05 N sodium hydroxide solution and then twice again with distilled water, and then allowed to crystallize. The crystals were removed, washed with water and dried. They were weighed and dissolved in enough methanol to give a total volume of 25 ml. Reaction mixtures for 5 per cent and 40 per cent of complete hydrolysis were prepared as before. The solvent was approximately 86 volume per cent methanol in each case. After 24 hours nearly all of the color had disappeared from the 5 per cent reaction tube. It was removed from the 25° water bath and treated as before except that the acid was washed twice with dilute hydrochloric acid and a sublimation, much slower than in the previous run, was carried out over a period of about eight hours.

After six days the phenolphthalein color still persisted in the 40 per cent reaction tube. When it was removed from the water bath and back-titrated with dilute hydrochloric acid, it was found to have reached 37 per cent of complete hydrolysis. This sample was treated as the 5 per cent hydrolysis had been.

The 100 per cent reaction mixture was refluxed for three days with three times the required amount of 1 N sodium hydroxide solution and allowed to stand for two more days at room temperature. It was then treated as the

other two samples had been.

In the radioassay of these last samples greater care was taken in getting a homogenous mixture of the ingredients for the zinc reduction and all of the determinations were made in one 24-hour period. Results are given in Table IV.

Data

TABLE III

ASSAY OF p-METHYL-t-BENZOIC ACID

Extent of Reaction	Activity	
	: After second : sublimation	: After third : sublimation
%	$\mu\text{c.}/\text{mmole}$	$\mu\text{c.}/\text{mmole}$
5	6.48	6.77
(RUN 1)	6.51	6.54
100	6.62 6.57 6.38	7.11 6.62 7.22

TABLE IV

ASSAY OF p-METHYL-t-BENZOIC ACID

Extent of Reaction	Activity	
	: Single : determinations	: Averages
%	$\mu\text{c.}/\text{mmole}$	$\mu\text{c.}/\text{mmole}$
5	5.45 5.44	5.448
(RUN 2)	5.45	
37	5.52 5.45	5.456
(RUN 3)	5.40	
100	5.51 5.53 5.55 5.49	5.522

Results

The ratios of reaction rate constants, calculated by using the equation of Stevens and Attree (17) and the data given in Table IV are given below.

run 2 k^*/k 0.986

run 3 k^*/k 0.985

DISCUSSION OF RESULTS

Statistical analysis of the data for the three samples whose activities were measured in runs 2 and 3 indicates that the true differences between the means of the activities of the acids between the 5 per cent and 100 per cent hydrolysis and between the 37 per cent and 100 per cent hydrolysis are as follows (6).

Between 5% and 100% Hydrolyses

Confidence Level	(Run 2)	True Difference
96%		between 0.098 and 0.050
98%		between 0.110 and 0.038

Between 37% and 100% Hydrolyses

	(Run 3)	
80%		between 0.108 and 0.024
90%		between 0.122 and 0.010

Although the data from the first set of hydrolyses (run 1) were not used in calculating a ratio of rate constants, they indicate an isotope effect in the same direction and of similar magnitude as shown in runs 2 and 3.

If the tritium atom had no effect other than to change the mass of the reacting particle, then theoretically the ratio of rate constants (k^*/k) would be equal to $\sqrt{u/u^*}$, where u is the reduced mass of the ester molecule and hydroxide ion, and the starred quantities are for the labeled

compound. The value calculated in this way is 0.999. The ratio of the rate constants which was found in this research has a deviation from unity several times as great as this value. Therefore it is necessary to explain the rate difference on the basis of difference in activation energies.

Since the reaction site is several atoms removed from the isotopic bond, the effect of a different isotope on the rate should be small. This is in agreement with the findings in this work.

The rate-determining step of the hydrolysis is thought to be the attack of the nucleophilic hydroxide ion on the carboxyl carbon. Therefore, an increase in the electron density about the carboxyl carbon would increase the required activation energy and decrease the reaction rate.

A p-methyl group can influence electron densities through a benzene ring by inductive and by conjugative effects. It is generally conceded that hyperconjugative resonance contributions are smaller for tritium and deuterium bonds than for their protium analog, due to their lower zero-point vibrational energies and the resulting bond stabilization. Therefore, if hyperconjugative resonance effects were the most important factor in governing the rate of hydrolysis and the other bonds of the methyl group were not altered by substitution of one tritium atom for protium, the isotopic molecule should hydrolyse faster in this reaction. However, this relationship was not found in this case.

If inductive effects are the governing factor and if the heavier isotope exhibits a greater positive inductive effect, as has been postulated for deuterium by Halevi (8), the isotope effect found in this research becomes readily understandable.

SUMMARY

The tritium isotope effect in the alkaline hydrolysis of methyl p-methyl-t-benzoate was measured.

The methyl ester of p-toluic acid which contained tracer amounts of tritium on the para methyl group was synthesized and hydrolysed in alkaline solution to known fractions of completion. Then by using the radioactivities of the reactant and of the acid which was produced, the ratio of reaction rate constants was calculated. The labeled compound was found to react 0.985 as fast as the unlabeled ester.

The exchange of tritium from tritiated water for the hydrogen atoms of the p-methyl group of p-toluic acid was found to be negligible at 100°.

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