A STUDY OF C^{13} AND C^{14} ISOTOPE EFFECTS IN SOME UNIDIRECTIONAL PROCESSES

A STUDY OF C^{13} AND C^{14} ISOTOPE EFFECTS

IN

SOME UNIDIRECTIONAL PROCESSES

By

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A study of C^{13} and, or, C^{14} isotope effects in some unidirectional chemical processes; additional evidence for the Cohen and Schneider mechanism for acid hydrolysis of tertiary butyl benzoate; a suggested mechanism for the decarboxylation of anthranilic acid; disagreement between theory and experiment for the ratio of the C^{13} and C^{14} isotope effects in the decarboxylation of mesitoic acid.

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TABLE OF CONTENTS

Contents Page

II HISTORICAL INTRODUCTION

2. Separation of Isotopes

3. Isotope Effects in Unidirectional Processes

4. Use of Isotope Effects in Mechanism Studies

5. Theory of Chemical Isotope Effects

III EXPERIMENTAL PROCEDURES

2. Preparation of Materials

Contents Page

IV RESULTS AND DISCUSSION

Contents Page

LIST OF FIGURES

LIST OF TABLES

I GENERAL INTRODUCTION

For many years after the discovery of isotopes, it was generally believed that the isotopes of an element possess identical chemical properties. Soon after the discovery of deuterium, it was found that the reactivity of this isotope is markedly different from that of ordinary hydrogen. Using the methods of statistical mechanics, and spectral data, Urey was able to make theoretical calculations of the equilibrium constants to be expected in equilibrium reactions involving hydrogen and deuterium. The extension of these methods to equilibria in which isotopes of heavier elements were involved showed that chemical separation of isotopes should be possible in many cases. Chemical exchange methods were then developed for the separation of the stable isotopes of carbon, nitrogen, oxygen, and sulphur, in particular. Later, evidence that fractionation processes occur in nature was found by careful mass spectrometric studies of the relative abundance of isotopes in samples of natural materials obtained from different sources.

Although the original view of the chemical identity of isotopes had thus undergone modification, the first observations of C^{13} and C^{14} isotope effects in unidirectional reactions were somewhat surprising because of the magnitude of the effects found. In 1948, Beeck and co-workers discovered that the dissociation probabilities of the C^{12} - C^{12} and the C^{12} - C^{13} bonds of the molecule-ions formed from propane - 1 - C^{13} by electron impact differed by about 20 per cent. Subsequently, they found that an 8 per cent greater frequency of rupture of $C^{12} - C^{12}$ than of $C^{12} - C^{13}$ bonds occurred when propane - 1 - C^{13} was subjected to thermal cracking. The first C^{14} isotope effect was reported shortly after by Yankwich and Calvin who found that the $C^{12} - C^{12}$ bond in C^{14}-carboxyl malonic acid ruptured about 12 per cent more frequently than the $C^{12} - C^{14}$ bond when this compound was thermally decarboxylated. When the corresponding bromomalonic acid was thermally decarboxylated, the bond rupture rates differed by 40 per cent.

These large isotope effects seemed to be of immediate importance in any research using C^{13} or C^{14} as tracers which attempted to obtain quantitative results. For this reason, a program of work was begun in the laboratories of the Atomic Energy Project, National Research Council, to determine the C^{14} isotope effect in a series of organic reactions so that chemists using this isotope might have knowledge of the occurrence and magnitude of the C^{14} isotope effect in unidirectional processes. Four reactions, alkaline hydrolysis of ethyl benzoate, the benzilic acid rearrangement, the benzoin condensation, and the Cannizzaro reaction were studied with this objective. Only the first of these is described in detail in the body of the thesis.

The original research plan was broadened, however, during the course of this work mainly as a result of the theory of relative reaction velocities of isotopic molecules which was developed by Bigeleisen.

Firstly, this theory brought a clearer understanding of the cause of isotope effects, of when they should occur, and of the magnitude of the effects to be expected. From the theoretical considerations of isotope effects, it seemed obvious that the occurrence, or lack of an isotope effect, and perhaps even the magnitude of the effect, should be useful in determining reaction mechanisms in certain cases. Deuterium isotope effects, which can be very large, had already been used for such purposes by Reitz, for example, in studying the enolization of acetone. One principle arising from the theory is that a large isotope effect should occur in bond cleavage only if an isotopic atom is at one end or the other of the bond being broken. It seemed that a study of the C^{14} isotope effect in alkaline and acid hydrolysis of C^{14}-carboxyl labeled tertiary butyl benzoate would be an interesting application, since the available chemical evidence indicates that the alkyl oxygen bond is broken in acid hydrolysis of tertiary esters whereas alkaline hydrolysis gives the normal acyl oxygen bond fission. A further example of the application of this basic principle in the elucidation of a reaction mechanism was found accidentally during a study of the C^{13} isotope effect in the decarboxylation of anthranilic acid. Both of these applications are described in this thesis.

Secondly, the theory of unidirectional isotope effects developed by Bigeleisen created an anomalous situation. The magnitude of the C^{14} isotope effect to be expected in the decarboxylation of malonic and bromomalonic acids as calculated by Bigeleisen was in sharp disagreement with the experimental values reported by Yankwich and Calvin. The 40 per cent effect observed in the bromomalonic acid case could be discounted because, as the authors stated, the material was impure. But the C^{14} effect in the malonic acid case still was high by a factor of about three. Experimental values of the C^{13} isotope effect in the same reaction obtained by Bigeleisen and Friedman, and by Lindsay, Bourns, and Thode agreed with theory. The inference was that the large C^{14} isotope effects observed were either due to poor technique or that large errors were inherent in the methods of measuring the C^{14} isotope effects. The work in this laboratory, as well as independent work by Brown and Holland, showed C^{14} isotope effects of the order of 10 to 14 per cent. These results appeared to discount the possibility of poor technique, and suggested that any errors inherent in measuring the C^{14} effects were at least fairly consistent. If the latter were true, it could be expected that the measured magnitude of C^{14} isotope effects would be the same, more or less independent of the experimenter. A more recent study of the C^{14} isotope effect in the decarboxylation of malonic acid - 1 - C^{14} by Roe and Hellman casts some doubt on this assumption, however. These authors reported a value of 1.06 ± .02 for the ratio of the decarboxylation rates which is to be compared with the values 1.12 ± .03 given by Yankwich and Calvin, and 1.04 calculated by Bigeleisen.

The general problem of C^{14} isotope effects was thus seen to be in a very unsatisfactory state. Only when theory explains experiment and experiment confirms theory can real progress in scientific knowledge, as opposed to empirical knowledge, be made. Experimental values for C^{14} isotope effects which could be trusted as true values should be able to make a considerable contribution to the theory of isotope effects, and to the closely related theory of absolute reaction rates. Present theory could either be confirmed, or the need for modification would be shown. For this reason a new approach to the measurement of C^{14} isotope effects was taken.

Measurements made with mass spectrometers normally have much higher precision than measurements of radioactivity. It therefore seemed logical to measure a C^{14} isotope effect using the more precise method, mass spectrometry. According to present theory, the C^{14} isotope effect in a given reaction should be very nearly twice the C^{13} isotope effect. With a mass spectrometer, both C^{13} and C^{14} effects could be measured at the same time using the same samples. Even in a reaction where theoretical calculation of the isotope effects would be difficult, if not impossible, measurement of both the C^{13} and C^{14} effects should yield valuable information.

The decarboxylation of mesitoic acid was chosen for such a study. The carboxyl carbon composition of the mesitoic acid used was approximately one per cent C^{14}, one per cent C^{13} and the rest C^{12}. This reaction was chosen both because the carbon dioxide produced in the reaction would be ideal for mass spectrometer analysis, and because Bigeleisen and Bothner-By had just completed a study of the C^{13} isotope effect occurring in this reaction.

To summarize, this thesis presents several studies of different aspects of the general problem of C^{13} and C^{14} isotope effects. A study of the C^{14} isotope effect in alkaline hydrolysis of C^{14}-carboxyl ethyl benzoate was undertaken to determine if a C^{14} isotope effect occurred in a typical ester hydrolysis. A study of the C^{14} isotope effects in acid and alkaline hydrolysis of tertiary butyl benzoate was made both to confirm the usefulness of the theory of isotope effects in mechanism studies, and to confirm the accepted mechanism for acid hydrolysis of this tertiary ester. A study of the C^{13} isotope effect in the thermal decarboxylation of anthranilic acid was made to obtain information which might be used in the elucidation of the mechanism of this reaction. In order to provide a more exact value of a C^{14} isotope effect for comparison with the C^{13} isotope effect in the same reaction, both the C^{13} and C^{14} isotope effects in the decarboxylation of mesitoic acid were measured using a mass spectrometer.

II HISTORICAL INTRODUCTION

1. Discovery of Isotopes

The concept of the homogeneous, indivisible atom of the eighteen-nineties was shattered by the discovery of radioactivity and the attendant research which followed in the first decade of the twentieth century. During the early studies of the radioactive group of elements, it was found that there were several instances where certain members of the group had identical chemical properties and, if mixed, could not be separated from one another by any known chemical reactions, although they were easily distinguishable by their differing radioactivities. Thus, thorium X, actinium X and radium formed one such group and ionium, radiothorium, radioactinium and thorium formed another. To express the idea that there were types of matter which were uniform in chemical behavior, but which could differ in other properties such as atomic weight and radioactivity, Soddy (69) introduced the word "isotope".

Sir J. J. Thomson's (81) investigations of the positive rays produced in X-ray tubes, and the work of Aston (1) with the mass spectrograph which followed, established the fact that isotopy was a feature of many of the stable elements, also.

The more recent development of the production of new atomic species by bombarding existing nuclei with simple particles such as neutrons, protons, and deuterons has increased the number of known isotopes of most elements and has even added new elements to the Periodic Table. Today, there is a vast array of nuclear species and many of these are available for individual study.

2. Separation of Isotopes

a. Physical Processes

The original concept of isotopes, as already noted and as implied in the Greek derivation of the word, was that they were members of a group having identical chemical properties. The mass spectrograph, in the very act of identifying stable isotopes, separates them by a physical process which depends on mass differences. Separation of weighable amounts of isotopes using the mass spectrograph principle was not achieved, however, until 1934 when the two isotopes of lithium, Li^6 and Li^7, were separated in microgram quantities by Oliphant, Shire, and Crowther (49). Other physical processes such as diffusion (2,34), thermal diffusion (19), fractional distillation (67), centrifugation (3), electrolysis (76), and adsorption (54) have been suggested and used with varying degrees of success to concentrate certain isotopes. Some of these physical processes have recently been developed to high degrees of efficiency in connection with atomic energy research, as is quite well known (68).

b. Chemical Processes

Chemical methods for separation, or concentration of isotopes were attempted as early as 1907 when Hoffman and Wölfl (36) claimed to have obtained a measurable separation of lead and radium D using the reaction:

$$2PbCl_2 + 4C_6H_5MgBr \longrightarrow Pb + (C_6H_4)_4Pb + 2MgCl_2 + 2MgBr_2$$

This claim was not substantiated by later workers (70,15,57), however, and the original concept of the chemical identity of the isotopes of an element was preserved until the discovery of a hydrogen isotope of mass two by Urey, Breckwedde, and Murphy (83), in 1932. As was expected in this unique case where one isotope is twice as heavy as another, even chemical properties were found to differ significantly. For this reason, it was natural to give the new isotope the separate name, deuterium. By the end of 1934, Farkas (28) was able to include a chapter on the chemistry of deuterium in his book "Light and Heavy Hydrogen". Equilibrium constants greater than three have been observed for exchange reactions involving hydrogen and deuterium. In rate studies, hydrogen has been found to react up to fifteen times as rapidly as deuterium. The chemical differences of the much more recently discovered radioactive hydrogen isotope, tritium, are even more pronounced.

Equilibrium reactions involving hydrogen and deuterium were first treated theoretically by Urey and Rittenberg (85) in 1933 using the methods of statistical mechanics and spectral data. Theory and experiment were found to be in very good agreement (86), for example, in the reaction:

$$2DI + H_2 \rightleftharpoons D_2 + 2HI \ .$$

The extension of the theory to isotopes of elements of higher atomic number by Urey and Grieff (84) showed that it should be possible to concentrate isotopes of some of the heavier elements using equilibrium reactions. Very shortly, Webster, Wahl, and Urey (88) found that in the equilibrium exchange reaction between water and carbon dioxide, the carbon dioxide was 3.5 per cent enriched in O^{18} at 25°C, confirming the theoretical prediction. Equilibrium exchange reactions were later developed for the concentration of N^{15} (80), C^{13} (58), and S^{35} (73), in particular. In a recent paper, Urey (82) has tabulated a large number of the isotopic exchange constants which have been calculated. Although only a few of these constants have been verified experimentally because of the difficulties in establishing the proper equilibrium reactions, the results which have been obtained leave no doubt as to the validity of the calculations.

Careful study of isotopic abundances of some of the lighter elements in naturally occurring materials from various sources has shown that considerable isotopic separation can occur in nature due to equilibrium, and probably unidirectional processes. Nier and Gulbransen (47), and Murphey and Nier (45), for example, found that the C^{12}/C^{13} ratio could range from 93.1 in Lycopodium spores, through 91.8 in wood, to 89.2 in limestone. Thode and co-workers (79,78) have found variations of up to 3.5 per cent in the abundance of the boron isotopes B^{10} and B^{11} and also variations of up to 5 per cent in the sulphur isotopes S^{32} and S^{34}.

3. Isotope Effects in Unidirectional Processes

a. Discovery.

Despite the gradual modification of the original view that isotopes of an element possess identical chemical properties, chemists using radioactive and stable isotopes as tracers generally considered the chemical differences to be insignificant for all practical purposes, except for hydrogen, deuterium and tritium. The first few reports of rather large carbon isotope effects in unidirectional processes were thus quite surprising.

The first carbon isotope effect to be observed in a unidirectional process occurred in a mass spectrometer analysis of C^{13} labeled propane. Beeck and co-workers (4) discovered that the dissociation of the $C^{12} - C^{12}$ bond in propane - 1 - C^{13} molecules, caused by electron bombardment in the mass spectrometer, had a 17 per cent greater probability of occurrence and the dissociation of the $C^{12} - C^{13}$ bond had a 12 per cent lesser probability of occurrence than the dissociation of a $C^{12} - C^{12}$ bond in a normal propane molecule. The overall effect gave a $C^{12} - C^{12}$ to $C^{13} - C^{12}$ bond rupture ratio of 1.22. Beeck and co-workers (73) followed this observation with a study of the isotope effect in the thermal cracking of propane - 1 - C^{13} at 500°C. The interpretation of the results is difficult because the mechanism of the reaction is not well understood, but the overall effect gave a $C^{12} - C^{12}$ to $C^{12} - C^{13}$ bond rupture ratio of 1.08 at 10 percent cracking, or 1.14 at infinitesimal cracking as calculated later by Bigeleisen (5).

Very shortly, Yankwich and Calvin (92) reported the first C^{14} isotope effect to be observed in a unidirectional process. They obtained a value of 1.12 for the $C^{12} - C^{12}$ to $C^{13} - C^{14}$ bond rupture ratio in the decarboxylation of C^{14}-carboxyl labeled malonic acid. The similar bond rupture ratio in the decarboxylation of C^{14}-carboxyl labeled bromomalonic acid was found to be 1.41, but the bromo acid was not pure and some doubt was attached to this very high value.

b. Further Experimental Determinations of C^{13} and C^{14} Isotope Effects

Very shortly after these C^{13} and C^{14} isotope effects were reported, a study of C^{14} isotope effects was begun in the Laboratories of the Atomic Energy Project, National Research Council. The first reaction studied was the alkaline hydrolysis of C^{14}-carboxyl labeled ethyl benzoate. Stevens and Attree (71) found that the ratio of the rates of hydrolysis for the C^{12}-carboxyl and the C^{14}-carboxyl ester was about 1.16, the C^{12}

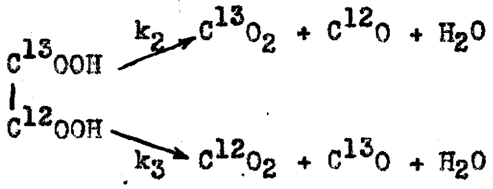
ester hydrolyzing more rapidly than the C^{14} ester. This work is described in the experimental section of this thesis.

Stevens and Attree (72) then studied the benzilic acid rearrangement of C^{14}-carbonyl benzil and found that the phenyl group joined to the C^{14} had a lower migration probability than the phenyl group joined to the C^{12}. The ratio of the amounts of benzilic acid - 2 - C^{14} and benzilic acid - 1 - C^{14} formed in the rearrangement was found to be about 1.11. They also reported that no appreciable C^{14} isotope effect was observed in either the benzoin condensation or Cannizzaro reaction of C^{14}-carbonyl labeled benzaldehyde.

While this work was in progress, reports of several C^{13} and C^{14} isotope effects which had been observed in different laboratories appeared in the literature. Daniels and Myerson (21) found that a C^{14} isotope effect occurred when labeled urea was hydrolyzed with the enzyme urease, the carbon dioxide produced toward the end of the reaction being depleted in C^{14}. The effect here was opposite to what might have been expected, but the reaction is complex. Another biochemically produced C^{14} isotope effect was reported by Weigl and Calvin (89) who found that photosynthesizing barley seedlings utilized $C^{12}O_2$ faster than $C^{14}O_2$.

Lindsay, McElcheran and Thode (40) studied the C^{13} isotope effect in the decomposition of ordinary oxalic acid in concentrated sulphuric acid. The two effects found were expressed quantitatively in terms of the three specific rate constants for the following equations:

$$(C^{12}O_2H)_2 \xrightarrow{k_1} C^{12}O_2 + C^{12}O + H_2O$$

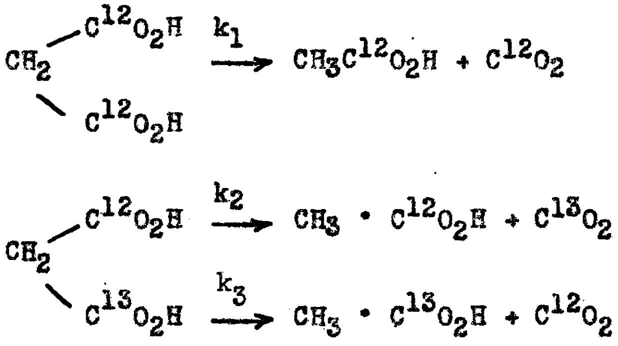


$$\begin{array}{l} C^{13}OOH \xrightarrow{k_2} C^{13}O_2 + C^{12}O + H_2O \\ | \\ C^{12}OOH \xrightarrow{k_3} C^{12}O_2 + C^{13}O + H_2O \end{array}$$

The values found were: $k_2/k_3 = 1.033$ and $k_1/(k_2 + k_3) = 1.034$.

c. Disagreement Between Theory and Experiment

About this time, Bigeleisen (6) presented theoretical calculations for the isotope effects to be expected in the thermal cracking of propane and the decarboxylation of malonic acid. The mechanism for the pyrolysis of propane, which is probably a chain reaction, is obscure. Since the isotope effect for only one possible elementary reaction was calculated by Bigeleisen, no correlation with experiment was possible for the propane reaction. The theoretical isotope effect for the malonic acid decarboxylation was in sharp disagreement with the experimental effect found by Yankwich and Calvin. Bigeleisen and Friedman (8) then studied the C^{13} isotope effect in the malonic acid decomposition using a mass spectrometer. Two isotope effects were discernable which were expressed quantitatively in terms of the specific rate constants for the following equation:



$$CH_2(C^{12}O_2H)_2 \xrightarrow{k_1} CH_3C^{12}O_2H + C^{12}O_2$$

$$CH_2(C^{12}O_2H)(C^{13}O_2H) \xrightarrow{k_2} CH_3 \cdot C^{12}O_2H + C^{13}O_2$$

$$CH_2(C^{12}O_2H)(C^{13}O_2H) \xrightarrow{k_3} CH_3 \cdot C^{13}O_2H + C^{12}O_2$$

The values found were: $k_1/2k_2 = 1.037$ and $k_3/k_2 = 1.020$. Their calculated values were: $k_1/2k_2 = 1.021$ and $k_3/k_2 = 1.0198$. Good agreement with theory was thus obtained for the ratio k_3/k_2 which expresses the intramolecular isotope effect. This was the effect measured by Yankwich and Calvin. Agreement with theory for the ratio $k_1/2k_2$, which expresses the intermolecular isotope effect, was very poor.

Pitzer (55), using the same basic theory as Bigeleisen, but a different molecular model, also calculated the C^{14} isotope effect to be expected in the malonic acid reaction and found good agreement with the experimental value of Yankwich and Calvin. Presumably his model would lead to poor agreement with the experimental value obtained for the C^{13} isotope effect by Bigeleisen and Friedman, since theory would predict that the C^{14} effect would be twice the C^{13} effect.

A more thorough study of the C^{13} isotope effect in the decarboxylation of ordinary malonic acid was made by Lindsay, Bourns and Thode (39). Substantial agreement with the effects found by Bigeleisen and Friedman was obtained.

Several other C^{14} isotope effects were then reported which seemed to be rather larger than would be expected on the basis of the theory developed by Bigeleisen. Evans and Huston (26) reported observing a large, but not accurately determined, C^{14} isotope effect in wet oxidation of acetic acid - 1 - C^{14}. They pointed out that acetic acid is somewhat resistant to oxidation by wet combustion and that quantitative results based on a wet combustion of labeled acetic could be in error if oxidation were not complete.

Weigl, Warrington, and Calvin (90) found that photosynthesizing barley seedlings took up $C^{14}O_2$ 17 per cent more slowly than $C^{12}O_2$. Several consecutive reactions, each having an isotope effect, are possibly involved here. Brown and Holland (18) measured the ratio of the rates of formation of the p-nitro-phenyl hydrazones of ordinary and C^{14} - carbonyl-benzophenone and obtained a value of about 1.10.

d. More Recent Experimental Determinations of C^{13} and C^{14} Isotope Effects.

An experimental value for a C^{14} isotope effect which does approach the theoretical value calculated by Bigeleisen was obtained by Roe and Hellman (59). These authors made a new study of the C^{14} isotope effect in the decarboxylation of malonic acid - 1 - C^{14} and reported a value of 1.06 ± .02 for the ratio of the two rate constants of the labeled molecule. This compares with the values 1.12 ± .03 found by Yankwich and Calvin and 1.04 calculated by Bigeleisen for the intramolecular effect.

Bothner-By and Bigeleisen (13) studied the relative rates of decomposition of carboxyl - C^{12} and carboxyl - C^{13} - mesitoic acids in sulphuric acid solution. The ratios of the rate constants at 61°C and 92°C were found to be 1.037 ± .003 and 1.032 ± .001, respectively. Bigeleisen and Allen (7) determined the relative rates of decomposition of carboxyl - C^{12} and carboxyl - C^{13} - trichloracetic acids. The ratio of the rate constants obtained at 70°C was 1.0338 ± .0007.

The results of a study of the C^{14} isotope effect in the dehydration of formic acid - C^{14} in 95 per cent sulphuric acid have been published since the completion of the experimental work contained in this thesis. Ropp, Weinberger, and Neville (60) found that C^{12} - formic acid decomposed 11.11 per cent faster than C^{14} - formic acid at 0°C and 8.59 per cent faster at 25°C. From these values it was calculated that a difference of 189 ± 53 calories per mole existed between the Arrhenius activation energies for the dehydration of the two acids.

Also since completion of the experimental work for this thesis, Fry and Calvin (30) have published results of further isotope effect studies in their laboratory. The C^{13} and C^{14} isotope effects occurring in the decomposition of oxalic acid in concentrated sulphuric acid were measured using a mass spectrometer to measure the C^{13} effect and an ionization chamber to measure the C^{14} effect. The intramolecular C^{13} isotope effect observed was in good agreement with the results obtained earlier by Lindsay, McElcheran, and Thode. The ratio between the C^{13} and C^{14} isotope effects was found to be very close to the theoretical value, two. These authors made some theoretical calculations and concluded that the observed effects were larger than would be expected from theory. A study of the C^{14} isotope effects in the decarboxylation of C^{14}-carboxyl labeled α-napthylmalonic and phenylmalonic acids was also made by Fry and Calvin (29). The ratio of the intramolecular decarboxylation rates was found to be 1.097 ± .008 for α-napthylmalonic acid - 1 - C^{14} at about 80°C, and 1.132 ± .015 for phenylmalonic acid - 1 - C^{14} at about 73°C. Both of these values were considered to be larger than present theory would predict.

4. Use of Isotope Effects in Mechanism Studies

a. General Discussion

Although theoretical calculations and experimental values of isotope effects are not as yet in good agreement, nevertheless the basic cause of chemical isotope effects seems to be understood. One conclusion which can be reached from the general theory is that a significant isotope effect should occur in bond cleavage only if the isotopic atoms are directly involved in the bond being broken. The presence of an isotopic atom in a molecule, in a position not involved in the bond cleavage, should have only a small, second order effect on the reaction rate. Thus the presence, or absence, of an isotope effect in a reaction may yield information as to whether a certain bond cleavage occurs or not, or whether it is rate determining.

b. Use of Deuterium Isotope Effects

The basic principles of isotope effects, as applied to deuterium reactions, were appreciated almost immediately after the discovery of this isotope. (75,87). The determination of the detailed mechanism of the enolization of acetone by Reitz (56) by the use of heavy water and deuteroacetone is a good example of the use of a deuterium isotope effect in a mechanism study. Quite recently Westheimer and Nicolaides (91) were able to show that the rate controlling step in the oxidation of isopropyl alcohol by chromic acid is the removal of the hydrogen on the secondary carbon atom, since 2 - deuteropropanol - 2 was found to be oxidized about one-sixth as fast as ordinary isopropyl alcohol in chromic acid solution.

b. Use of Other Light Element Isotope Effects

Since the discovery of large unidirectional isotope effects for C^{13} and C^{14}, and the development of the theory of relative reaction velocities of isotopic molecules by Bigeleisen, carbon, oxygen and nitrogen isotope effects have been used for the determination of reaction mechanisms in several cases.

Bothner-By, Bigeleisen, and Friedman (13) studied the pyrolysis of ordinary barium adipate to form cyclopentanone and found that the C^{13} isotopic fractionation in this process was only about 0.1 per cent. They suggested that the very small effect could be explained if the direction of cyclization was dictated by the preliminary removal of a proton from one of the α carbons, a process which would be subject to only a very slight isotope effect as far as the carboxyl groups are concerned.

Bigeleisen and Friedman (9) used the results of their study of N^{15} and O^{18} isotope effects in the decomposition of ammonium nitrate to further the knowledge of the mechanism of this reaction. Bourns, Stacey, and Lindsay (14) used the results of their study of the N^{15} isotope effect in the deamination of phthalamide to phthalimide as the basis for a mechanism which they proposed for this reaction. Fry and Calvin (30) were able to suggest a mechanism for the decomposition of oxalic acid from a consideration of the results of their study of the C^{13} and C^{14} isotope effects which occurred in that reaction.

5. Theory of Chemical Isotope Effects

a. General Discussion

The motion of the electrons about an assemblage of nuclei in a molecule depends only on the electric field of the nuclei. The fields about isotopic atoms, and hence about isotopic molecules, are almost certainly so nearly the same that they may be considered identical.

The electronic states and the potential field in which the nuclei are moving will thus remain the same if isotopic substitution is made in a molecule. The force constants between atoms therefore depends on their atomic numbers and not on their atomic masses and the chemical potential of molecules is independent of their isotopic composition.

On the basis of classical statistical mechanics, where kinetic energy follows the equipartition law and the distribution of the molecules in space is given by the Boltzmann equation, there should be no chemical isotope effect in equilibrium reactions. Classical theory would allow a very small isotope effect in unidirectional reactions of higher order than the first, because the collision numbers for molecules is a function of their masses. Chemical isotope effects of considerable magnitude <u>do</u> occur, both in equilibrium and unidirectional processes, however. These isotope effects are quantum phenomena.

The energy, E, of a molecule may be considered to be the sum of two independent terms, the translational energy, E_{tr}, and the internal energy, E_{int}. The latter term may be further expressed as a sum of electronic energy, E_e, rotational energy, E_r, and vibrational energy, E_v. Thus,

$$E = E_{tr} + E_{int} = E_{tr} + E_e + E_r + E_v$$

All of these energies are quantized in quantum mechanics. However, the energy levels for translational energy are so closely spaced that the distribution can be considered as continuous and hence, classical. For most chemical reactions, the electrons are all in their ground states and only for a very few polyatomic molecules and various free radicals, which have a multiplet ground state, does the electronic energy have to be considered. Corresponding electronic states for isotopic molecules are in any case almost identical, as stated earlier. If the product of absolute temperature, T, and the Boltzmann constant, k, is large compared with the separation of rotational energy levels, as it generally is, except at very low temperatures, or for compounds of hydrogen, the rotational energy level distribution can also be considered to be classical. The vibrational energy levels, however, are relatively far apart and higher levels than the ground state may be occupied at moderate temperatures. The vibrational energy is the only type of internal energy to be affected by quantum considerations, normally, and it is the source of chemical isotope effects.

The vibrational energy of a diatomic molecule, assuming that it behaves as a harmonic oscillator, which is very nearly true, is $(n + 1/2) h\nu$, according to quantum mechanics. In this expression, n is the quantum number and may be zero or any integer, h is Planck's constant, and ν is the fundamental vibration frequency of the molecule in its ground state. The frequency of such a harmonic oscillator is a function of the masses, m_1 and m_2, of the two adjacent atoms and is given by the formula:

$$\nu = \frac{1}{2\pi} \sqrt{f/\mu} ,$$

where f is the force constant between the atoms and μ is the reduced mass, defined as $\frac{1}{\mu} = \frac{1}{m_1} + \frac{1}{m_2}$. The vibrational energy situation is more complicated for polyatomic molecules, but the energy levels of each normal mode of vibration can be calculated in a similar way.

The vibrational energy of a molecule, being dependent on frequency, is therefore a function of the masses of the atoms which constitute the molecule. In the lowest energy level, where the quantum number is zero, there will be an internal energy difference between two isotopic diatomic molecules of $1/2\ h\ (\nu_1 - \nu_2)$, where ν_1 and ν_2 are the vibrational frequencies for the light and heavy molecules respectively. This energy difference is known as the zero-point energy difference. The situation for polyatomic molecules, though more complex, is essentially the same.

Molecular vibrations are not strictly harmonic, and the anharmonicity increases as the quantum number increases. At high temperatures, the corresponding energy levels of isotopic molecules approach the same values. The internal energy difference between two isotopic molecules will thus, in general, be some large fraction of the zero-point energy difference.

b. Equilibrium Processes.

Chemical equilibrium obtains when the energy of a reacting chemical system is at a minimum. When isotopic molecules are involved in an equilibrium process, the final distribution of isotopic atoms amongst the molecules will be governed by energy considerations. Since there are differences in the internal energies of isotopic molecules, and since the differences related to particular isotopic atoms are not constant from one molecular species to another, it is to be expected that the distribution of isotopic atoms will not be statistical, as to number, amongst the various molecular species. Chemical isotope effects in equilibrium are thus due to differences in the vibrational energies of isotopic molecules.

c. Unidirectional Processes

In isotopic molecules, the force constants are practically identical as stated earlier. The absolute energy level at which two chemically bound atoms may be separated should therefore be independent of their isotopic structure. However, since a light molecule contains more vibrational energy than a corresponding heavy isotopic molecule, the light molecule will require less additional energy for bond rupture than the heavy molecule. The difference in energy will be, as has been shown, some large fraction of the zero-point energy difference, if the molecules are at some ordinarily encountered temperature.

Most chemical reactions have a temperature dependence given by the Arrhenius equation,

$$k = A\ e^{-E/RT}$$

where k is the reaction rate, A is a constant over a reasonably large temperature range and E, the so-called "activation energy", is a measure of the energy which a molecule must acquire, over and above its ground state energy, in order to react. The ratio of reaction rates of two isotopic molecules should thus be:

$$k_1/k_2 = e^{-(E_1 - F_2)/RT}$$

where $(E_1 - E_2)$ is, to a first approximation, equal to the zero-point energy difference between the two isotopic molecules, $1/2h(\nu_2 - \nu_1)$. Chemical isotope effects in unidirectional processes are thus also, to a large extent at least, traceable to differences in the vibrational energies of the isotopic molecules.

d. The Vibrational Energy Partition Function

The probability that a molecule will have energy ϵ_i in any quantum state that is g_i-fold degenerate is proportional to the quantity $g_i e^{-\epsilon_i/kT}$, where k is the Boltzmann constant and T is the absolute temperature. This is the Maxwell-Boltzmann distribution law and may be used unless the temperature is very low or the pressure very high. The total probability of occurrence of a molecular species in <u>any</u> energy state, which is then the probability of its existence, is proportional to $\sum_i g_i e^{-\epsilon_i/kT}$. The total number of molecules, N, in a given volume is thus also proportional to $\sum_i g_i e^{-\epsilon_i/kT}$ and this summation, often designated by "Q", is known as the "partition function" or "state sum". All thermodynamic quantities such as equilibrium constants, free energies and entropies may be expressed in terms of the partition functions of the molecules or atoms involved.

It will be realized from the earlier discussion that the partition function of most interest here is the vibrational energy partition function,

$$Q_v = \sum_i g_i e^{-\epsilon_{i\,vib}/kT}$$

where $\epsilon_{i\ vib}$ is the difference between the vibrational energy in any level and the lowest level. The vibrational energy for diatomic molecules is given by

$$\epsilon_v = (n + 1/2)h\nu$$

When n = o, the zero-point vibrational energy, ϵ_o, is

$$\epsilon_o = 1/2\ h\nu$$

We thus obtain

$$\epsilon_{vib} = (\epsilon_n - \epsilon_o) = nh\nu$$

and the vibrational partition function is

$$Q_v = \sum_{n=0}^{\infty} e^{-nh\nu/kT} = (1 - e^{-h\nu/kT})^{-1}$$

For polyatomic molecules having x atoms, the vibrational partition function is normally

$$Q_v = \prod_{i=1}^{3x-6} (1 - e^{-h\nu_i/kT})^{-1}$$

the product being taken over all 3x - 6 (or 3x - 5, for linear molecules) modes of vibration.

If the minimum of the potential energy curve of the vibrational energy of a molecule rather than the zero point energy is chosen as the reference zero, the two partition functions become

$$Q_v = \frac{e^{h\nu/2kT}}{1-e^{-h\nu/kT}}$$

and

$$Q_v = \prod_{i=1}^{3x-6} \frac{e^{-h\nu_i/2kT}}{1-e^{-k\nu_i/kT}}$$

e. Calculation of Isotope Effect in Exchange Equilibria

Urey (82), in his recent paper on the thermodynamic properties of isotopic molecules, has discussed the detailed mathematical calculation of equilibrium constants for isotopic exchange reactions. Bigeleisen and Mayer (10) also published a paper about the same time giving substantially the same theoretical treatment. The latter paper is valuable because it presents some mathematical short-cuts in the calculations.

A typical exchange reaction may be written

$$A_1 + B_2 \rightleftharpoons A_2 + B_1$$

where A and B are molecules which have some element in common and the subscripts 1 and 2 indicate that molecule 1 has a light isotope and molecule 2 has a heavy isotope. The equilibrium constant for this reaction is given by

$$K_c = \frac{[A_2] \times [B_1]}{[A_1] \times [B_2]} \quad \ldots\ldots\ldots\ldots \quad (1)$$

where the square brackets indicate concentrations. The number of molecules of any species contained in a given volume is, as has been stated, proportional to the complete partition function of that species in the given volume. Equation (1) may therefore be written

$$K_c = \left(\frac{Q_{A_2}/V}{Q_{A_1}/V}\right)\left(\frac{Q_{B_1}/V}{Q_{B_2}/V}\right) \quad \ldots\ldots\ldots\ldots \quad (2)$$

$$= \left(\frac{Q_{A_2}}{Q_{A_1}}\right) \bigg/ \left(\frac{Q_{B_2}}{Q_{B_1}}\right) \quad \ldots\ldots\ldots\ldots \quad (3)$$

The ratio Q_2/Q_1, which is the ratio of the partition functions of two isotopic molecules, is given rigorously by the equation

$$\frac{Q_2}{Q_1} = \frac{\sigma_1}{\sigma_2}\left(\frac{M_2}{M_1}\right)^{3/2} \frac{\sum e^{-\epsilon_2/kT}}{\sum e^{-\epsilon_1/kT}} \times \frac{f(I_2)}{f(I_1)} \quad \ldots\ldots\ldots\ldots \quad (4)$$

where σ_1 and σ_2 are the symmetry numbers for the two isotopic molecules, M_1 and M_2 are their molecular weights and ϵ_1 and ϵ_2 are corresponding energy states for the two molecules. The summations extend over all energy states and are calculated from the minimum of the potential energy curves of the molecules, i.e. the hypothetical vibrationless state. If the temperature is sufficiently high so that the rotational energy can be considered classical, and if the vibrations are sufficiently close to harmonic, equation (4) may be replaced by

$$\frac{Q_2}{Q_1} = \frac{\sigma_1}{\sigma_2} \quad \frac{I_2}{I_1} \left(\frac{M_2}{M_1}\right)^{3/2} \cdot \frac{e^{-u_2/2}}{e^{-u_1/2}} \cdot \frac{(1 - e^{-u_1})}{(1 - e^{-u_2})} \quad \ldots\ldots \quad (5)$$

for diatomic molecules, and

$$\frac{Q_2}{Q_1} = \frac{\sigma_1}{\sigma_2} \left(\frac{A_2B_2C_2}{A_1B_1C_1}\right)^{1/2} \quad \frac{M_2}{M_1}^{3/2} \prod_{i=1}^{3\lambda-6} \left\{ \frac{e^{-u_{2_i}/2}}{e^{-u_{1_i}/2}} \quad \frac{(1 - e^{-u_{1_i}})}{(1 - e^{-u_{2_i}})} \right\} \quad (6)$$

with no internal Rotation.

for polyatomic molecules. The I's are the moments of inertia for the diatomic molecules and the A's, B's and C's are the principle moments of inertia of the polyatomic molecules. For convenience, u replaces $h\nu/kT$.

According to a theorem of Teller and Redlich (77),

$$\frac{I_2}{I_1} \cdot \left(\frac{M_2}{M_1}\right)^{3/2} \cdot \left(\frac{m_1}{m_2}\right)^{3/2n} \cdot \frac{u_1}{u_2} = \left(\frac{A_2B_2C_2}{A_1B_1C_1}\right)^{1/2} \cdot \left(\frac{M_2}{M_1}\right)^{3/2} \cdot \left(\frac{m_1}{m_2}\right)^{3/2n} \prod_i \frac{u_{1i}}{u_{2i}} = 1$$

where m_1 and m_2 are the atomic weights of the isotopic atoms being considered in the reaction and n is the number of isotopic atoms being exchanged.

Hence, if equation (5) is multiplied on both sides by

$(m_1/m_2)^{3/2n}$, and if the right side is multiplied by and divided by u_1/u_2, a simplification results and we obtain

$$\frac{Q_2'}{Q_1'} = \frac{Q_2}{Q_1} \cdot \left(\frac{m_1}{m_2}\right)^{3/2n} = \frac{\sigma_1}{\sigma_2} \cdot \frac{u_2}{u_1} \cdot \frac{e^{-u_2/2}}{e^{-u_1/2}} \cdot \frac{1 - e^{-u_1}}{1 - e^{-u_2}} \quad \ldots\ldots\ldots \quad (5^1)$$

If equation (6) is multiplied on both sides by $(m_1/m_2)^{3/2n}$, and if the right side is multiplied by and divided by $\prod_i u_{1i}/u_{2i}$ a similar simplification results and we obtain

$$\frac{Q_2'}{Q_1'} = \frac{Q_2}{Q_1} \cdot \frac{m_1}{m_2}^{3/2} = \frac{\sigma_1}{\sigma_2} \cdot \prod \frac{u_{2i}}{u_{1i}} \cdot \frac{e^{-u_{2i}/2}}{e^{-u_{1i}/2}} \cdot \frac{1 - e^{-u_{1i}}}{1 - e^{-u_{2i}}} \quad \ldots\ldots \quad (6^1)$$

The equilibrium constant, K, is then given by

$$K = \left(\frac{Q'_{A_2}}{Q'_{A_1}}\right) \Big/ \left(\frac{Q'_{B_2}}{Q'_{B_1}}\right) \quad \ldots\ldots\ldots\ldots\ldots \quad (3^1)$$

and can thus be calculated from the fundamental vibration frequencies of the two isotopic molecules provided these have been observed in Raman or infra-red spectra studies, or have been calculated.

For isotopic exchange reactions where the differences in the vibrational frequencies of the isotopic molecules is such that $(h\nu_1/kT) - (h\nu_2/kT) = u_1 - u_2 = \Delta u$ is small, Bigeleisen and Mayer (10) have shown that the equilibrium constant may be calculated from the formula

$$\ln K = \sum_i \left(\frac{1}{2} - \frac{1}{u_i} + \frac{1}{e^{u_i-1}}\right) \Delta u_i - \ln \frac{\sigma_2}{\sigma_1}$$

$$= \sum_i G(u_i)\ \Delta u_i - \ln \frac{\sigma_2}{\sigma_1} \quad \ldots\ldots\ldots\ldots\ldots \quad (7)$$

where $G(u_i) = (1/2 - \frac{1}{u_i} + \frac{1}{e^{u_i}-1})$, and they have tabulated values of $G(u_i)$ from $u = o$ to $u = 25$.

It must be realized, of course, that the equations given above have been developed for perfect gas reactions and include assumptions such as that the vibrations are harmonic and the rotational energy may be considered as classical. Various corrections have to be made to apply the theory to condensed phase reactions and to hydrogen and deuterium exchange reactions. The aim of this discussion has been merely to outline the general method by which theoretical calculations for equilibrium constants of isotopic exchange reactions can be made.

f. Calculation of Isotope Effects in Unidirectional Processes.

Provided that the rate determining step in a chemical process, $A + B + C + \ldots \longrightarrow$ Products, is the surmounting of an energy barrier, the rate constant, according to the Eyring (27,31) method, is given by the formula

$$k = K \frac{C^{\ddagger}}{C_A C_B C_C \ldots} \left(\frac{kT}{2\pi m^{*}}\right)^{1/2} \frac{1}{\delta} \quad \ldots\ldots\ldots \quad (1)$$

K is the transmission coefficient, C_A, C_B, C_C ... are the concentrations of reactants A, B, C ..., $C^{\ddagger}$ is the concentration of the activated complex lying in the length, δ, of the decomposition coordinate, and k is the Boltzmann constant. The effective mass, m^{*}, of the complex in the direction of the decomposition coordinate, is normally taken as the reduced mass of the two atoms between which a chemical bond is being formed or broken.

For two isotopic molecules A_1 and A_2, where the subscripts indicate that molecule 1 has the lighter isotopic atom and 2 the heavier, two rate equations may be written as

$$k_1 = K_1 \cdot \frac{C_1^{\ddagger}}{C_{A_1} C_B C_C \cdots} \cdot \left(\frac{kT}{2\pi m_1^*}\right)^{1/2} \cdot \frac{1}{\delta_1} \quad \cdots\cdots \quad (2)$$

$$k_2 = K_2 \cdot \frac{C_2^{\ddagger}}{C_{A_2} C_B C_C \cdots} \cdot \left(\frac{kT}{2\pi m_2^*}\right)^{1/2} \cdot \frac{1}{\delta_2}$$

The potential energy surfaces for isotopic molecules are practically identical, as stated previously, so that $\delta_1 = \delta_2$ for our purposes here. It would be desirable to be able to calculate K_1 and K_2, but failing that, it appears (35) that K_1 will be equal to K_2 to a first approximation.

The ratio of the reaction rates for two isotopic molecules is thus

$$\frac{k_1}{k_2} = \frac{C_1^{\ddagger}}{C_2^{\ddagger}} \cdot \frac{C_{A_2}}{C_{A_1}} \cdot \left(\frac{m_2^*}{m_1^*}\right)^{1/2} \quad \cdots\cdots\cdots\cdots\cdots \quad (4)$$

The ratios of concentrations may be replaced by corresponding ratios of partition functions, treating the activated complex as an ordinary molecule. The reaction rate ratio is then

$$\frac{k_1}{k_2} = \frac{Q_1^{\ddagger}}{Q_2^{\ddagger}} \cdot \frac{Q_{A_2}}{Q_{A_1}} \cdot \left(\frac{m_2^*}{m_1^*}\right)^{1/2} \quad \cdots\cdots\cdots\cdots\cdots \quad (5)$$

where the Q's are the <u>complete</u> partition functions. It is most convenient to take the minimum of the potential energy curve for the molecules as the zero of the energy scale for the molecules. The minimum in the saddle of the potential energy surface of the activated complex may be taken as the zero for calculation of the partition function for the activated complexes.

Bigeleisen and Mayer (10) have defined a function f as

$$f_A = \frac{Q_{A_2}}{Q_{A_1}} \prod_i \left(\frac{M_{i1}}{M_{i2}}\right)^{3/2} \quad \ldots\ldots\ldots\ldots (6)$$

where the M_i's are the masses of the isotopic atoms in the isotopic molecules. The function, f, has been evaluated by them and is a function of the molecular vibrations only. They obtain

$$f = \frac{\sigma_1}{\sigma_2} \prod_{i=1}^{3n-6} \frac{u_i}{u_i + \Delta u_i} \; e^{\Delta u_i/2} \cdot \frac{1 - e^{-(u_i + \Delta u_i)}}{1 - e^{-u_i}} \quad \ldots (7)$$

The σ's are the symmetry numbers, $u_i = h\nu_i/kT$ and $\Delta u_i = h(\nu_{i1} - \nu_{i2})/kT$, as in the equilibrium equations given earlier. A similar relation holds for $f^{\ddagger}$, the corresponding function for the activated complexes. The ratio of the reaction rates, in terms of the function f, from (5) and (6), becomes

$$\frac{k_1}{k_2} = \frac{f}{f^{\ddagger}} \left(\frac{m_2^{\ddagger}}{m_1^{\ddagger}}\right)^{1/2} \quad \ldots\ldots\ldots\ldots (8)$$

When Δu_i is small, as it is for ordinary temperatures and all molecules except compounds of hydrogen and its isotopes,

$$\frac{k_1}{k_2} = \frac{\sigma_1}{\sigma_2} \frac{\sigma_2^{\ddagger}}{\sigma_1^{\ddagger}} \left(\frac{m_2^{\ddagger}}{m_1^{\ddagger}}\right)^{1/2} \left[1 + \sum^{3n-6} G(u_i)\Delta u_i - \sum^{3n'-6} G(u_i^{\ddagger})\Delta u_i^{\ddagger}\right] \ldots (9)$$

where $G(u) = (1/2 - 1/u + 1/(e^u - 1))$, as before. It might be noted that both $\sum G(u_i)\Delta u_i$ and $\sum G(u_i^{\ddagger})\Delta u_i^{\ddagger}$ have positive values, or are zero, in the general case.

According to quantum mechanics, there is a definite probability that some molecules will succeed in getting from the initial to the final state without having to surmount the energy barrier. This effect, known as "tunneling", depends on the shape of the energy barrier and can be calculated. It is usually neglected since there does not yet appear to be a single chemical reaction where the importance of the "tunnel effect" has been demonstrated with certainty.

The chief difficulty in employing formulae (7) and (8), or formula (9), is that the properties of the activated complex are extremely difficult, if not impossible, to determine. However, the equations should yield qualitative information. If the vibration frequencies of the isotopic molecules in question are known, or can be calculated with some accuracy, the equations should give an upper limit to an isotope effect, also.

In equation (9), (m_2^*/m_1^*) is always unity or greater than unity. In bond cleavage reactions, where the isotopic atoms are directly involved, $\sum^{3n-6} G(u_i)\Delta u_i$ will normally be greater than $\sum^{3n'-6} G(u_i^{\ddagger})\Delta u_i^{\ddagger}$. In this type of reaction, the rate constant for the light molecule will be greater than the rate constant for the heavy molecule. In bond formation reactions, where the isotopic atoms are directly involved, $\sum^{3n'-6} G(u_i^{\ddagger})\Delta u_i^{\ddagger}$ can be greater than $\sum^{3n-6} G(u_i)\Delta u_i$. If $\sum^{3n'-6} G(u_i^{\ddagger})\Delta u_i^{\ddagger}$ is greater than $\sum^{3n-6} G(u_i)\Delta u_i + 1/2 \ln (m_2^*/m_1^*)$, the rate constant for the heavy molecule will be greater than that for the light molecule. In the case of reactions of isotopic <u>atoms</u>, where $\sum^{3n-6} G(u_i)\Delta u_i$ is zero since there are no vibrations initially, the rate constant for the heavy atom may be greater than that for the light atom, because, although $(m_2^*/m_1^*) > 1$, $\sum^{3n'-6} G(u_i^\ddagger)\Delta u_i^\ddagger$ may be sufficiently large to nullify the first effect. This condition occurs in reactions of the hydrogen isotopes and $kT > kD > kH$.

The largest values for the rate ratios will occur when the isotopic atoms are essentially free, or vibrationless, in the activated complex. In this case, $\sum^{3n'-6} G(u_i^\ddagger)\Delta u_i^\ddagger$ approaches zero and the reaction rate ratio is given by

$$\frac{k_1}{k_2} = \frac{\sigma_1}{\sigma_2}\,\frac{\sigma_2^\ddagger}{\sigma_1^\ddagger}\left(\frac{m_2^*}{m_1^*}\right)^{1/2}\left(1 + \sum^{3n-6} G(u_i)\,\Delta u_i\right)$$

III EXPERIMENTAL PROCEDURES

1. General Precautions

All volatile C^{14} compounds were handled in a hood having good ventilation. The vacuum lines and racks used were in enclosed hoods. Powders were usually handled in a "dry box". Surgical rubber gloves were worn when working with active compounds. A special effort was made at all times to avoid spills, spattering of liquid droplets, or creation of dusts containing active material. Vessels, trays, blotting paper and Kleenex were arranged for each operation so that any accidental spills, large or small, could be controlled and would not result in contamination of the laboratory or other experimental materials. Used glassware was always given a preliminary wash in the hood with an appropriate solution, solvent, or detergent. A more thorough washing using a detergent and careful rinsing followed. For critical experiments, glassware which had never been used for any radiochemical work was used.

No radiation hazard existed because C^{14} has a very soft β emission, 154 k.e.v., which is nearly all absorbed by a few centimetres of air, or by an ordinary thickness of glass.

Although the recognized maximum tolerance level for C^{14} in the air was 3×10^{-2} microcuries per cubic metre when this work was begun, this has since been put as high as one microcurie per cubic metre for continuous exposure. The health hazard was thus not very great and control of contamination was more important for experimental than for health reasons.

2. Preparation of Materials

a. Benzoic Acid-Carboxyl-C^{14}

Various techniques for preparing carboxyl labeled acids by carbonation of the appropriate Grignard reagent with labeled carbon dioxide have appeared in the literature. (33,61,23). The chief difference from an ordinary Grignard carbonation is that in making a carboxyl labeled acid, the carbon dioxide is usually the more valuable chemical. An excess of Grignard is used therefore, rather than the reverse, and the carbon dioxide is handled in a vacuum system of one kind or another. Benzoic acid-carboxyl-C^{14} has been prepared by Dauben, Reid and Yankwich (24).

Several different preparations of benzoic acid-C^{14} having various activities were made at different times. The source of the C^{14} was either dry barium carbonate-C^{14} or, more frequently, a solution of sodium carbonate-C^{14} with excess sodium hydroxide present. Dry barium carbonate is very sensitive to electrostatic charge and it is quite difficult to transfer the powder without creating a dust. Sodium carbonate solution is quite convenient since aliquots may be readily taken and transferred with a pipette. The water may be readily removed from the solution in the following manner: An aliquot of solution is pipetted, using a rubber bulb, or a pipette fitted with a ground glass syringe, into a tube about 17 mm. in diameter and 20 cm. in length. This tube is attached to a vacuum line by means of a ground glass joint and the solution is frozen using a dry ice-cellosolve bath. The tube is then evacuated and the vacuum line isolated from the pumps by turning a stop-cock. By placing a liquid nitrogen bath around a second evacuated tube attached to the vacuum line and carefully warming the tube containing the solution with an infra-red lamp, the water is distilled without the solution melting and is condensed in the cold tube. The sodium carbonate, which contains some sodium hydroxide, is finally dried by being heated to about 150°C while still under high vacuum.

A typical preparation of benzoic acid-C^{14} was as follows: Lead chloride (2 to 3 g.) was added to the tube containing the dry sodium carbonate-C^{14} (3 millimoles) obtained as described above, or to dry barium carbonate weighed into a similar tube. The tube was attached to the vacuum line and evacuated to 10^{-3} mm., or less. The vacuum line was isolated from the pumps and the lead chloride-sodium carbonate mixture was heated to about 350°C, at which temperature the mixture melts and evolves dry carbon dioxide at a moderate rate. The carbon dioxide was condensed into a small liquid nitrogen cooled, storage bulb attached to the vacuum line. When gas evolution was complete, the bulb was isolated from the vacuum line by turning a stop-cock. Phenylmagnesium bromide (10 millimoles) was prepared in a 50 ml. round bottomed flask. This flask was then attached to the vacuum line by means of a ground glass joint, the Grignard reagent frozen by surrounding the flask with a liquid nitrogen bath, and the flask evacuated. The flask was isolated from the vacuum line by turning a stop-cock and the Grignard was allowed to melt. The Grignard was then refrozen in liquid nitrogen and the flask re-evacuated to a pressure less than 10^{-3} mm. The vacuum line was isolated from the pumps and the carbon dioxide storage bulb was opened to the line. The carbon dioxide was allowed to thaw and was then frozen in the Grignard flask. The Grignard was allowed to thaw with the flask open to the vacuum line, and was stirred with a magnetic stirrer. When the temperature of the reaction mixture had risen to about 10°C, the flask was again immersed in liquid nitrogen to freeze the ether and carbon dioxide. The Grignard flask was then isolated from the vacuum line by turning a stop-cock. The mixture was allowed to thaw and was then stirred for about one half hour with the flask immersed in a dry ice-cellosolve bath kept at about -10°C. The flask was then removed from the vacuum line and the Grignard addition product decomposed by adding chipped ice and dilute sulphuric acid. The reaction mixture was then rinsed into a separatory funnel and extracted with ether[*]. The ether extract was washed with water and then extracted with excess 1 N sodium hydroxide solution. The benzoic acid was precipitated from this solution by the addition of 6 N hydrochloric acid. The product, almost pure white, was removed by filtration using a sintered glass filter funnel. This material was diluted with a desired amount of pure benzoic acid and the whole amount was then twice recrystallized from hot water, using a small quantity of bone charcoal in the first recrystallization. The acid was dried

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[*] Dow-Corning silicone stop-cock grease was found to be ideal for lubricating the stop-cock and the stopper of funnels used for active solutions. A small amount of the grease was also placed on the outside of the tip of the funnel to prevent solution creep. The separatory funnel used here, and others used throughout the work, was a modified type having the original one-way stop-cock replaced by a three-way stop-cock. This modification allowed the liquid layers to be cleanly separated without the top layer having to be poured through the top of the funnel.

and stored in a vacuum desiccator over silica gel. Yields in dummy runs were 80 to 85 per cent, based on the carbon dioxide; m.p. 120.5 - 121.5°C.

b. Ethyl Benzoate-Carboxyl-C^{14}

Ethyl benzoate-carboxyl-C^{14} was prepared through the acid chloride. Benzoic acid (10 millimoles) was heated with an excess of thionyl chloride (25 millimoles) in a 25 ml. flask. When the evolution of hydrogen chloride ceased, the excess thionyl chloride was removed at slightly reduced pressure, using a small fractionating column. An excess of absolute alcohol (40 millimoles) was added and the mixture was refluxed for two hours. The excess alcohol was removed at atmospheric pressure. Pure inactive ethyl benzoate (5 ml.) was added and the product was subjected to fractional distillation at reduced pressure. A large middle fraction, $n_D^{25} = 1.5057$, was collected and used in the isotope effect experiments.

c. Tertiary Butyl Benzoate-Carboxyl-C^{14}.

This ester was made according to the method given by Norris and Rigby (48) and later used by Cohen and Schneider (20). Benzoic acid-carboxyl-C^{14} (30 millimoles) and thionyl chloride (60 millimoles) were heated under reflux in a 25 ml. flask for an hour. The excess thionyl chloride was removed by heating the mixture at 100°C for a half hour. Pyridine (60 millimoles) and tertiary butyl alcohol (45 millimoles) were added and the mixture was allowed to stand for 24 hours with the flask stoppered. Water (10 ml.) was added to dissolve the pyridine hydrochloride and the contents of the flask were washed into a separatory funnel with a little ether. The ether layer was washed once with five per cent sodium carbonate solution, twice with small portions of water, and finally dried over anhydrous sodium sulphate. The ether was then removed and the residue distilled through a small column to give an 80 per cent yield of ester, b.p. 112°C at 18 mm.; $n_D^{25} = 1.4897$.

d. Anthranilic Acid

Eastman Kodak Co.'s "Eastman" grade anthranilic acid was recrystallized twice from hot water using a little bone charcoal in the first recrystallization. The acid was dried and stored in a vacuum desiccator over silica gel; m.p. 146-147°C.

e. Bromomesitylene

Bromomesitylene was prepared according to the directions given in "Organic Syntheses" (52) using one-tenth the quantities given there. A colourless product was obtained; b.p. 106-108°C at 18-20 mm. Yield was 66 per cent of the theoretical amount.

f. Mesitoic Acid-Carboxyl-C^{13},C^{14}

A modification of the method described in "Organic Syntheses" (51), carbonation of the Grignard obtained from bromomesitylene, was used to prepare mesitoic acid-carboxyl-C^{13}, C^{14}.

In making the Grignard from bromomesitylene, better results were obtained with an activated magnesium. A solution of 0.5 ml. of methyl iodide in one ml. of ether was poured over magnesium (40 millimoles) in a 50 ml. flask. As soon as reaction started, the methyl iodide solution was poured off and the magnesium was washed with two 10 ml. portions of dry ether. Bromomesitylene (40 millimoles) was diluted with 10 ml. of dry ether and a small portion of this solution was added to the magnesium. Reaction was started by gently warming the flask. The rest of the bromomesitylene solution was slowly added to the magnesium while the flask was warmed with a Glass-col mantle to keep the reaction mixture refluxing gently. After about four hours, the magnesium had nearly all disappeared and the reaction was considered to be complete. The carbon dioxide-C^{13},C^{14} for this preparation was obtained in essentially the same way as described for the benzoic acid preparation. The water was removed from a sodium carbonate solution containing about eight millicuries of C^{14} and inactive anhydrous sodium carbonate was added to make a total amount of ten millimoles. Lead chloride (6g.) was added and the carbon dioxide was generated and frozen in a storage bulb on the vacuum line. The carbonation of the Grignard was carried out as described previously in the benzoic acid preparation. The carbonated Grignard reagent was rinsed into a separatory funnel containing about 50 g. of cracked ice and 6 ml. of concentrated hydrochloric acid, using 25 ml. of ether. After the Grignard had decomposed, the aqueous layer was run into a second separatory funnel and was extracted a second time with 25 ml. of ether. The combined ether extracts were washed with water and then extracted with excess 1 N sodium hydroxide solution. The alkaline solution was warmed to remove ether. The solution was then acidified with concentrated hydrochloric acid and the mesitoic acid precipitated as pale yellow crystals. The mother liquor was removed by filtration using a small sintered glass filter stick. The mesitoic acid was then twice recrystallized from dilute aqueous alcohol. The dummy inactive run gave a yield of 1.3 g. of acid, or 79 per cent of the theoretical yield, based on carbon dioxide; m.p. 151-152°C. In the active run, 0.5 g. of pure inactive mesitoic acid was added to the active acid before the second recrystallization. The yield of active acid was 1.8 g. The acid was vacuum dried and stored in a vacuum desiccator over silica gel.

3. Measurement of C^{14} Radioactivity

a. General Considerations

The determination of the C^{14} isotope effect in a reaction requires a knowledge of the change in the concentration of the molecules containing C^{14} which occurs as a result of the reaction. Since only the percentage change in concentration need be known, a parameter directly proportional to the concentration of C^{14} may be used instead of the absolute concentration.

If a defined area of a layer of material containing C^{14} is placed near a Geiger or a proportional counter, so that the β-particles produced by disintegration can enter the active volume of the counter, the recorded disintegration rate will be directly proportional to the amount of C^{14} contained in the material, within a known probable error range, which depends on the number of counts recorded. This measurement, which can be expressed in some such form as "counts per minute per millimole", will thus serve as a suitable parameter, provided that the proportionality constant can be made the same for all samples. In practice, it is impossible to fulfill this latter requirement strictly, for two main reasons. If the samples to be compared are not identical as to mass thickness, density, and surface characteristics, the proportionality constants will be different because the C^{14} β-particles have a low energy and are self-absorbed to a considerable extent in the material being counted. The counting rate also varies with time due to changes in the background count and to changes in the characteristics of the counting device being used.

The first difficulty may be largely overcome in three different ways. If sufficiently thick layers, i.e. layers of 25 mgm. per $cm.^2$, or thicker, are used, self-absorption reaches a maximum and becomes constant. For thinner layers, counting rates may be determined for various thicknesses of material and the self-absorption determined. The counting rate for all samples may then be corrected to some standard thickness. If sufficiently thin layers are used, self-absorption becomes negligible. The second difficulty is largely overcome by taking frequent background counts and by frequently counting a standard sample. The background count is subtracted from the observed count for all samples and all sample counts are corrected for any drift which is shown by the standard sample. Furthermore, samples may be recounted in any order and on different days.

b. Sample Preparation

Thin samples require the least amount of radioactive material and, when they can be used, provide the most direct way of obtaining the desired measurement of radioactivity. The method of thin sample preparation was therefore chosen for the radioactivity measurements made for the work described in this thesis. The material which had to be counted was benzoic acid. It was obtained from the various experiments, finally, as a solution containing known amounts of sodium benzoate per millilitre. It was found that the sodium salt could not be plated in thin layers sufficiently uniform to give the desired reproducibility in the counting results. The ammonium salt was known to give more uniform samples and this fact suggested the use of the hydrazine salt. This salt appeared to give very uniform layers, but the counting results were erratic. The difficulty was traced to decomposition of the salt and sublimation of the benzoic acid under the drying lamp. However, it was found that the sodium salt could be plated to give quite reproducible counting results if a little hydrazine hydrate was present in the solution. Apparently, the addition of this material lowered the surface tension of the solution slightly and increased the solubility of the sodium salt sufficiently, so that solid material did not precipitate out until the solution volume was very small, and when precipitation did occur, the solid did not tend to go to the outer edge and form a ring.

The method of sample preparation finally adopted was as follows: One tenth ml. of hydrazine hydrate solution (80 percent by volume) was added to the sodium benzoate solutions before they were finally diluted to 25 ml. in volumetric flasks. Samples of 50 λ, 100 λ or 200 λ of these solutions were pipetted with "Misco"* pipettes onto stainless steel discs. The discs were 2.5 cm. in diameter, having an area of

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* Made by "Microchemical Specialty Co"., Berkeley, California.

3.45 cm.^{2} defined by a thin, 2 mm. wide, ring of "Tygon" paint primer around the outside rim. Since the samples were counted using a counter which was insensitive to relatively large changes in area of the sample, the samples did not have to be spread over the entire area of the disc, but an effort was made to have the solution spread over at least half of the area available. The important factor was to obtain uniform thickness and avoid rings and ridges. The samples were dried under an infra-red lamp, with a gentle stream of air directed toward the disc. Just before the samples were dry, the discs were picked up with forceps and the solution was gently swirled. If the latter operation was done at the appropriate moment, the solution then flash dried after being replaced under the lamp for a few moments.

Samples which appeared to be unevenly spread generally gave counts which, in extreme cases, were low by as much as 10 per cent, compared to well spread samples. Those poorly spread samples could be brought into solution with a few drops of one-half per cent aqueous hydrazine solution and then redried. The count on such respread samples generally rose to the value obtained for the well spread samples. Six or more samples for counting were made from each sodium benzoate solution.

Sample thicknesses ranged from 0.05 to 0.2 mgm. per cm.^{2}, the actual thickness depending on the solution sampled, the sample volume and the area covered. Self-absorption was considered to be negligible at these thicknesses since a constant counting rate per milligram of material was obtained when the sample thickness was varied by a factor of four, in this range, for any particular solution sampled.

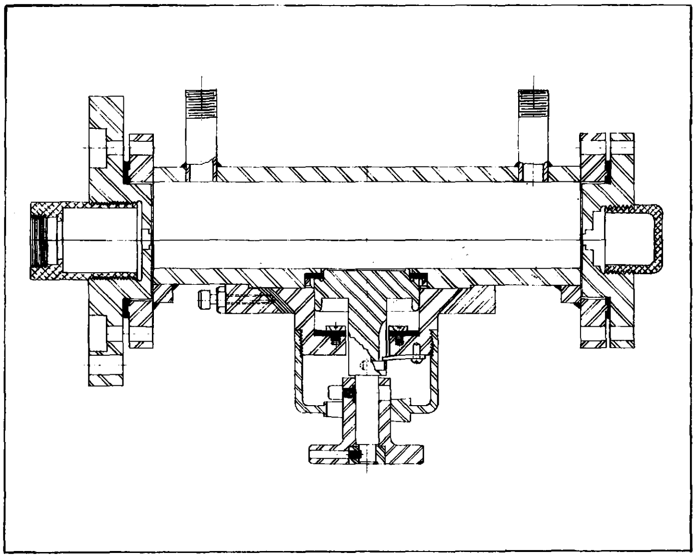


Figure 1. Counter assembly

c. Counting Apparatus

The samples were counted in a Simpson (66) type, methane filled, proportional counter designed and built at the Atomic Energy Project, National Research Council. The counter tube is shown in section in Figure 1. The counter is essentially a stainless steel tube, 4 cm. x 16 cm., having two removable end plates which support a tungsten wire. The two thousandths, or one thousandth, inch diameter wire is insulated from the end plates by polystyrene plugs and carries the positive high potential. The tube itself is grounded. Small inlet and outlet pipes at the top are provided for the methane supply. The sample holder consists of a horizontal sliding platform having a vertical sliding solid cylinder with a circular indentation for the steel sample disc.

The power unit for the counter, designated as "Power Unit, Type 200", was manufactured by Dynatron Radio Limited, Maidenhead, England, as was the scaler which was designated as "Scaling Unit, Type 200". A linear amplifier "Model 204-C" and a head amplifier "Model 204-A" manufactured by Atomic Instrument Company, Boston, Massachusetts, were run in series between the counter and scaler. The line voltage was stabilized with a Sola transformer. Matheson commercial methane, 96 per cent methane, was used as the counter filling.

The main advantages of this type of counter over the standard, thin, end-window type of Geiger tube for C^{14} counting are as follows: The large absorption of the soft β-particles by the mica window and by the air between the sample and the window is absent since the sample is inside the tube. The sample is very close to the wire and the geometry is such that practically all β-particles emerging from the top of the sample are counted regardless of their initial emission direction. The counter is therefore effectively a 2π counter and the counting efficiency is very nearly 50 per cent. Since the samples are mounted on steel, back-scattering of the β-particles increases the counting efficiency to slightly greater than 50 per cent. A counter having a high counting efficiency allows low activities to be counted more accurately and more rapidly than a counter having a low counting efficiency. When the Simpson type of counter is used in the proportional range with proper linear amplifier gain, feed back and pulse discrimination, the plateau, which extends over 250 volts or more, is longer, and generally has a flatter slope, than the plateau of the usual Geiger tube. This feature makes the counting rate less sensitive to line voltage fluctuations. Since the filling of the tube in continually being renewed, the plateau does not continuously deteriorate as does the plateau of a sealed Geiger tube. Since the tube has a complete metallic envelope, it does not become light sensitive as do many end-window Geiger tubes.

There are a few disadvantages, however. The tungsten wire, being under considerable tension, is liable to break. Since the samples are placed inside, the tube is rather easily contaminated. Replacement of the wire and decontamination of the tube are relatively simple, but time-consuming operations. The tube requires a voltage of about 3,000 volts for a two thousandths inch diameter wire, which voltage is more difficult to supply than the usual 1,000 to 1,200 volts required for an argon-alcohol filled Geiger tube. The operation of the Simpson type tube requires a linear amplifier which the Geiger tube operation does not.

d. Counting Procedure

Sample discs were placed in the circular depression in the sample holder cylinder, the sample holder was slid horizontally into position, and the cylinder was slid vertically up against a rubber gasket and locked into a fixed position. A small amount of air entered the tube during this operation, but it was sufficiently well flushed out in fifteen to twenty seconds by the methane flow that counting could be started. Samples were normally counted long enough to give a total number of counts close to 10,000, so that the standard error in the individual counting rate for any particular sample was approximately

$$r = \pm \frac{100 \sqrt{10{,}000}}{10{,}000} = \pm 1 \text{ per cent}$$

Many sample discs were counted more than once. The average counting rate was then taken as the activity value for the sample disc. The radioactivity of a particular fraction of benzoic acid was taken as the average activity of the six or more individual samples of that fraction counted. The error formula applied was that for the standard error of the mean value of a series of observations,

$$r = \sqrt{\frac{\sum_{1}^{n} d^2}{n(n-1)}}$$

where r is the standard error of the mean, d is the deviation of a single observation from the mean, and n is the number of observations.

Background was counted for two or three minutes several times during any series of sample counts. The background normally ranged from 50 to 60 counts per minute for the unshielded counter, but could be brought down to about 20 counts per minute by 2 inches of lead shielding. The background in the counting room was continuously recorded with an Esterline-Angus Graphic Ammeter so that any wide or sudden variations were always known.

4. Alkaline Hydrolysis of Ethyl Benzoate-Carboxyl-C^{14}

The experimental procedure used to determine the C^{14} isotope effect in the alkaline hydrolysis of ethyl benzoate-carboxyl-C^{14} at room temperature was, in brief, as follows: A sample of the ester was allowed to undergo hydrolysis in dilute aqueous alcohol containing excess sodium hydroxide until about 10 per cent of the ester was hydrolyzed. The reaction mixture was then divided into two equal portions and a sample of the benzoic acid which had been produced during the partial hydrolysis was isolated from one half of the solution. The hydrolysis was allowed to go to completion in the second half and a sample of the benzoic acid produced by complete hydrolysis was isolated. The specific activities of these two benzoic acids were determined by counting samples as already described. The values for these specific activities, and the value for the fraction of the ester which had hydrolyzed in the partial hydrolysis, supplied the necessary information for calculation of the ratio of the rates of hydrolysis for the normal ester molecules and the molecules containing C^{14}.

The detailed procedure was as follows: A 1.3 ml. sample of ethyl benzoate-carboxyl-C^{14} was pipetted into a 100 ml. volumetric flask and sufficient 0.5 N aqueous-ethanol (50% by volume) sodium hydroxide solution was added to make a total volume of 100 ml. The contents of the flask were well mixed. After about two minutes, at which time it was known that about 10 per cent of the ester would be hydrolyzed, a

50 ml. sample of the solution was withdrawn and pipetted into a 500 ml. separatory funnel containing 200 ml. of distilled water and 75 ml. of ether. (The ether used for extraction in this and in subsequent work was washed with dilute, acidified ferrous sulphate solution and then with distilled water just prior to use, to remove peroxides). The separatory funnel was gently shaken and the two layers were allowed to separate. The aqueous layer, containing the sodium benzoate produced in the hydrolysis, was run into a second separatory funnel containing 75 ml. of ether, and a second extraction was performed. The ether layer in the first funnel, containing the unhydrolyzed ester, was run into a storage flask. The aqueous layer from the second extraction was run into a third separatory funnel and extracted a third time with 50 ml. of ether. Ample time was allowed for good separation of the aqueous and ether layers to occur in all extractions. The aqueous layer was then run into a fourth separatory funnel and was acidified by the dropwise addition of 6 ml. of 6 N sulphuric acid. The benzoic acid liberated was extracted with 75 ml. of ether and the aqueous layer was then discarded. The ether extract containing the benzoic acid was washed with two 20 ml. portions of water, allowing ample time for separation of the layers. The ether solution was finally run through the unused side of the two-way stopcock on the separatory funnel into a 250 ml. Frlenmeyer flask. This flask was fitted with a stopper having an inlet and an outlet tube. A stream of dry nitrogen was allowed to enter the inlet tube, and the outlet tube was connected to a water pump, by means of which a slight vacuum was maintained. The flask was placed in a warm water bath to aid in the evaporation of the

ether. When the ether had been removed, the benzoic acid was dissolved in 15 ml. of alcohol. Two samples of this solution, of about 5 and 10 ml. respectively, were pipetted into 25 ml. volumetric flasks and were titrated with 0.05 N sodium hydroxide using phenol red indicator. The alcohol used had a negligible blank, one drop of the base being sufficient to change the colour of the indicator. Hydrazine hydrate was added to each flask as described under sample preparation, and the solutions were made up to 25 ml. with distilled water.

At the same time that the 50 ml. of solution was withdrawn from the main solution, a 5 ml. sample was withdrawn by a co-worker. This sample was pipetted into a separatory funnel, containing 20 ml. of water and 20 ml. of ether, simultaneously as the 50 ml. sample was pipetted into the larger separatory funnel. The aqueous layer was run into a flask and the ether layer was washed with 10 ml. of water, which was then added to the aqueous layer in the flask. This solution was immediately titrated with approximately 0.1 N sulphuric acid using phenol red indicator. From the normality of this aliquot, the normality of the original sodium hydroxide solution used for hydrolysis, and the normality of the second half of the hydrolysis solution after complete hydrolysis, the extent of hydrolysis at sampling time was determined. Although the accuracy of this determination left something to be desired, the value obtained was considered to be sufficiently accurate for the purpose of the experiment, since the calculated ratio of reaction rates does not depend very critically on the value for the fraction hydrolyzed.

The second half of the hydrolysis solution was allowed to stand for a week to complete hydrolysis and a 6 ml. sample was then withdrawn.

The benzoic acid was isolated from this sample by ether extraction after acidification with sulphuric acid. The ether solution was washed and the benzoic acid was isolated, divided, titrated, and made up into two 25 ml. sodium benzoate solutions as described above for the acid obtained from partial hydrolysis of the ester.

In the isolation of the benzoic acid samples, great emphasis was placed on obtaining pure samples and no attempt was made to obtain quantitative recoveries. After counting of samples was completed, each sodium benzoate solution was acidified with dilute sulphuric acid. The benzoic acid liberated in each solution was then extracted with 50 ml. of ether. Each ether solution was washed twice with 20 ml. portions of water, and the benzoic acid in each was isolated, titrated, and again made up into a sodium benzoate solution for recounting. This procedure provided an indication of the reliability of the specific activity determinations.

At a considerably later date, alkaline hydrolysis of ethyl benzoate-carboxyl-C^{14} was repeated in connection with the work on acid and alkaline hydrolysis of tertiary butyl benzoate-carboxyl-C^{14} and acid hydrolysis of ethyl benzoate-carboxyl-C^{14}. The same general method was followed. A smaller sample of ester, 500 λ, was used however and the hydrolyzing solution used was 55 percent alcohol by volume. This change was made because of the low solubility of the tertiary butyl ester. Since only about 40 per cent as much benzoic acid was produced, because of the smaller quantity of ester used, the benzoic acid recovered was not divided into two fractions as before. After total hydrolysis, a 5 ml. sample of the main solution was taken, and again the benzoic acid isolated from this sample was not divided. Duplicate runs were made.

5. Alkaline Hydrolysis of Tertiary Butyl Benzoate-Carboxyl-C^{14}

The experimental procedure used to determine the C^{14} isotope effect in alkaline hydrolysis of tertiary butyl benzoate-carboxyl-C^{14} was practically identical to the second procedure described for alkaline hydrolysis of the ethyl ester. A 500 λ sample of tertiary butyl benzoate-carboxyl-C^{14} was pipetted into a 100 ml. volumetric flask and the volume was made up to 100 ml. with a solution 55 per cent by volume alcohol and 0.5 N with respect to sodium hydroxide. The ester was allowed to hydrolyze at room temperature for seven hours, at which time it was known that about 10 per cent of the ester would be hydrolyzed. The procedure followed for isolating the benzoic acid produced and for preparing a solution for counting was then the same as described in the last section.

To obtain a sample of benzoic acid from completely hydrolyzed ester, the following method was adopted. A 5 ml. sample of the hydrolyzing solution was pipetted into a 50 ml. Erlenmeyer flask and 5 ml. of 6 N sodium hydroxide solution and 5 ml. of absolute alcohol were added. It was found necessary to use the more concentrated sodium hydroxide solution, since hydrolysis in 0.5 N alkali was very slow. This mixture was allowed to stand for twelve days. (Preliminary experiments had shown that hydrolysis was complete in seven to eight days under these conditions). The solution was then rinsed into a separatory funnel containing 100 ml. of water and 50 ml. of ether. The aqueous solution was acidified by the addition of 6 ml. of 6 N sulphuric acid and the benzoic acid liberated was then isolated, titrated, and made up into a sodium benzoate solution for counting as previously described.

The extent of partial hydrolysis was determined as follows: Two 5 ml. samples of the hydrolyzing solution were taken at the same time as the 50 ml. sample, i.e. seven hours after the start of hydrolysis, and were titrated directly with approximately 0.1 N sulphuric acid using phenol red indicator. The fraction of ester hydrolyzed was then obtained from the normality of these samples, the normality of the original alkaline hydrolysis solution, and the calculated weight of the 500 λ sample of ester used.

6. Acid Hydrolysis of Tertiary Butyl Benzoate-Carboxyl-C^{14}

A 500 λ sample of tertiary butyl benzoate-carboxyl-C^{14} was placed in a 100 ml. volumetric flask. The volume was made up to 100 ml. with a solution 55 per cent by volume ethyl alcohol and 1.6 N with respect to sulphuric acid. The mixture was allowed to hydrolyze for 28 hours, at which time it was known that about 10 per cent of the ester would be hydrolyzed. A 50 ml. sample was then removed and pipetted into a separatory funnel containing 54 ml. of 1.6 N sodium hydroxide solution, 46 ml. of water and 50 ml. of ether. The benzoic acid produced by hydrolysis was then isolated and made up into a sodium benzoate solution for counting as previously described.

To obtain a sample of benzoic acid from completely hydrolyzed ester, the procedure used for the total alkaline hydrolysis was adopted. A 5 ml. sample of the acid hydrolysis mixture was pipetted into a 50 ml. Erlenmeyer flask and 5 ml. of 6 N sodium hydroxide solution and 5 ml. of absolute alcohol were added. This mixture was allowed to stand for thirteen days. It was then rinsed into a separatory funnel containing 100 ml. of water and 50 ml. of ether. Extraction of the benzoic acid and preparation of a sodium benzoate solution for counting followed the usual procedure. Duplicate runs were made.

7. Acid Hydrolysis of Ethyl Benzoate-Carboxyl-C^{14}

Acid hydrolysis of ethyl benzoate was found to be very slow under the conditions used for acid hydrolysis of the tertiary butyl ester. The hydrolysis was therefore done at an elevated temperature, instead of at room temperature. A 500 ml. sample of ethyl benzoate was pipetted into a 100 ml. volumetric flask and made up to 100 ml. with the dilute alcoholic sulphuric acid solution used previously. The solution was then transferred to a pressure flask, and the flask was stoppered and placed in a water bath at 58.5 ± 0.5°C. After thirteen and one half hours, at which time approximately 10 per cent of the ester was hydrolyzed, the flask was removed from the water bath and cooled. A 50 ml. sample was withdrawn and the benzoic acid isolated in the usual manner.

To obtain a benzoic acid sample from totally hydrolyzed ester, 5 ml. of the acid hydrolysis solution was pipetted into a 50 ml. Erlenmeyer flask and 5 ml. of 6 N sodium hydroxide was added. After a week, the benzoic acid was isolated and the usual sodium benzoate solution prepared for counting. Duplicate runs were made.

8. Mass Spectrometry

a. Apparatus

The mass spectrometer used in this investigation was a 90°-

deflection instrument which, except for several modifications, was similar to one previously described by Graham, Harkness, and Thode (32). In the present instrument, the tube pumping system employed a two-stage fractionating oil diffusion pump, (Distillation Products Type GF-20W), operating on octoil-S and backed by a Welch Type 1405H forepump. This pumping system was used in conjunction with an automatic liquid air controller (43) which kept the mass spectrometer tube free of all oil vapour peaks and allowed a pressure of lower then 1 x 10^{-7} mm. Hg. to be maintained consistently in the tube, as indicated by an ion gauge (Distillation Products Type DPA-38).

The positive ion source was of the type recently described by Nier (46). The gas to be analyzed was introduced at 2.5 cms. Hg. pressure through a capillary leak into the ionization chamber of the source, and ionized by collision with an intense beam of electrons of 50 electron-volts energy. The positive ions were collimated into an ion beam of 2000 electron-volts energy by the collimating plates of the source, and directed normally into a square-shaped magnetic field of 2000 gauss. This magnetic field resolved the ion beam into its various mass components and deflected the central beam through a mean radius of curvature of 15 cms. The central beam, of one mass component, was focussed through the collector slit onto the collector plate. The collector plate was surrounded by an electrostatic field of -45 volts (from a dry B-battery) so that secondary electrons formed by positive ion bombardment of the collector plate were repelled back to the plate. The ion current was applied to the grid of a 954 electrometer tube (with a grid leak resistor of 10^{11} ohms) feeding a stabilized linearfeedback direct-current amplifier. Mass spectra were obtained by automatic magnetic scanning and were recorded by a Leeds and Northrup Type G Speedomax.

b. Analyses

Each analysis of a carbon dioxide gas sample consisted of six to ten pairs of spectrograms, a pair of spectrograms being obtained by scanning from mass 44 to 46, and then in the reverse direction from 46 to 44. The arithmetic mean of the two values of each ion current peak in each spectrogram was determined, and from these mean values, the ratio of ion currents was calculated.

The C^{13}/C^{12} ratio for each sample was obtained from the mass spectrometer ratio of mass 45 ion current peak to mass 44 ion current peak, by correcting for the contribution of $C^{12}O^{16}O^{17}$ to the mass 45 peak as follows:

$$\frac{(45)}{(44)} = \frac{C^{13}O^{16}O^{16} + C^{12}O^{16}O^{17}}{C^{12}O^{16}O^{16}} = \frac{C^{13}}{C^{12}} + \frac{O^{16}O^{17}}{O^{16}O^{16}}$$

$$\therefore \frac{C^{13}}{C^{12}} = \frac{(45)}{(44)} - \frac{O^{16}O^{17}}{O^{16}O^{16}}$$

Since all of the CO_2 samples were equilibrated with water from the same source before analysis, the ratio $O^{16}O^{17}/O^{16}O^{16}$ was the same for all samples, and this correction was determined to be 0.000802 from the measured oxygen abundances of the equilibration water.

The C^{14}/C^{12} ratio was obtained similarly from the mass spectrometer ratio of mass 46 peak to mass 44 peak, correcting for unwanted contributions to the mass 46 peak as follows:

$$\frac{(46)}{(44)} = \frac{C^{14}O^{16}O^{16} + C^{13}O^{16}O^{17} + C^{12}O^{16}O^{18} + C^{12}O^{17}O^{17}}{C^{12}O^{16}O^{16}}$$

$$= \frac{C^{14}}{C^{12}} + \frac{C^{13}}{C^{12}} \times \frac{O^{16}O^{17}}{O^{16}O^{16}} + \frac{O^{16}O^{18}}{O^{16}O^{16}} + \frac{O^{17}O^{17}}{O^{16}O^{16}}$$

$$\frac{C^{14}}{C^{12}} = \frac{(46)}{(44)} - \frac{C^{13}}{C^{12}} \times \frac{O^{16}O^{17}}{O^{16}O^{16}} - \frac{O^{16}O^{18}}{O^{16}O^{16}} - \frac{O^{17}O^{17}}{O^{16}O^{16}}$$

The oxygen ratio corrections were the same for all analyses and were as follows: $\frac{O^{16}O^{17}}{O^{16}O^{16}} = 0.000802$, $\frac{O^{16}O^{18}}{O^{16}O^{16}} = 0.00425$ and $\frac{O^{17}O^{17}}{O^{16}O^{16}} = 1.6 \times 10^{-7}$. The particular C^{13}/C^{12} ratio correction was obtained from the corresponding (45)/(44) ratio which was determined as described above.

Although individual values of the isotopic ratios may vary over a period of time, they can be determined with a precision of ± 0.1 per cent by comparing each sample with a standard. In this work, tank CO_2, purified by repeated distillation, was used as a standard, and each of the CO_2 samples was compared with it.

c. Reliability

Various factors affecting the reliability of the mass spectrometer results were given careful consideration and a discussion of these factors follows:

(i) Mass Resolution: - The familiar equation which gives the radius of curvature of an ion moving in a magnetic field may be expressed as

$$m/e = 4.82 \times 10^{-5} H^2 r^2 / V$$

where m is the mass of the ion in atomic mass units, e the number of electronic charges on the ion, H the magnetic field intensity in gauss, r the radius of curvature of the ion path in cms., and V the energy of the ion in volts. From the above equation it can be shown that the resolution of the mass spectrometer, $\Delta m/m$, is equal to $2 \Delta r/r$. The quantity $2\Delta r$ may be evaluated from the sum of the widths of the entrance and exit slits of the analyzer tube. In the instrument used, $\Delta m/m = (0.025 + 0.050)/15.0 = 1/200$. Hence, because the mass resolution was one mass unit in 200, the ion peaks were completely resolved, and no corrections had to be made for incomplete resolution.

(ii) Mass Discrimination: - From the above equation, it may be seen that either H or V may be changed to bring the ion beam of desired mass to focus on the collector. When H is changed, magnetic scanning is obtained, and when V is changed, electrostatic scanning is obtained. It has been shown (42) that mass discrimination in the 90° mass spectrometer accompanies electrostatic scanning, but is very much less with magnetic scanning. In this work, mass spectra were scanned magnetically to reduce this mass discrimination.

(iii) Ion Current Discrimination: - The direct current amplifier was a linear amplifier, and the linearity of the recording system has been established by Lossing, Shields, and Thode (41). The fact that consistent and accepted values of any given isotopic ratios have been obtained whether high or low ion currents were measured indicated that there was no non-ohmic response of the input grid leak resistor in the voltage range in which it was used. Any non linearity of this kind would largely cancel out anyway, when measuring small differences between samples.

(iv) Secondary Electron Emission: - Bombardment of the collector plate by positive ions results in the emission of secondary electrons from the plate, and this emission, unless supressed, may account for as much as one third of the measured peak height. A voltage of -25 volts on the suppressor plate, however, is sufficient to suppress this emission. Since -45 volts was applied to the suppressor, it was concluded that the secondary electron contribution to the measured ion currents was negligible.

(v) Isotopic Fractionation at the Capillary Leak: - Gas flow through the capillary leak is viscous, and no isotopic fractionation is to be expected. In this investigation, it was found that there was no change in the measured isotopic abundances even after the gas had been passing through the leak for seventy-two hours.

(vi) Purity of Samples: - The samples were purified by repeated vacuum distillations. The almost complete absence of masses, other than those arising from CO_2, in the spectrograms was evidence of the purity of the samples.

(vii) Contamination by Residuals: - Except for mass 18 (H_2O) and mass 28 (N_2), there were no residual foreign ions in the spectrometer tube in the mass range 10 to 100. Thus there was no evidence of contamination of the samples by residual gases in the mass spectrometer.

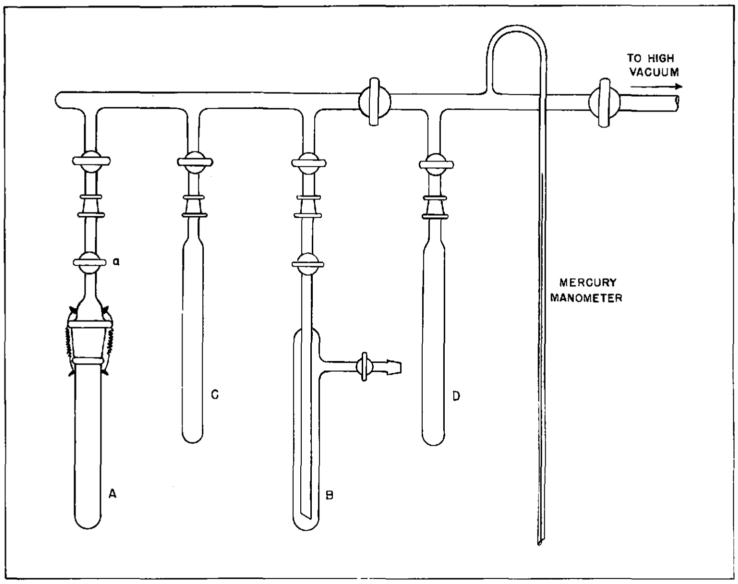


Figure 2. Vacuum line

9. The Decarboxylation of Anthranilic Acid

The method used to determine the C^{13} isotope effect in the decarboxylation of anthranilic acid was the same, in principle, as that used to determine the C^{14} isotope effect in ester hydrolysis. Essentially, the C^{13} concentration in the carbon dioxide produced during partial decarboxylation of a sample of anthranilic acid was compared with the C^{13} concentration in the carbon dioxide produced by complete decarboxylation of a sample of the acid. A mass spectrometer was used to measure the relative abundances of C^{12} and C^{13} in the carbon dioxide samples.

a. Thermal Decarboxylation

Pawlewski (53) found that anthranilic acid decarboxylated on being heated above its melting point to give aniline and carbon dioxide.

Since anthranilic acid sublimes very readily, and since the carbon dioxide produced in the decarboxylation was required for mass spectrometer analysis, the method adopted for thermal decomposition was as follows: In a typical run, 50 to 150 mg. of acid was placed in tube A, approximately 40 ml. in volume. The tube was then attached to a high-vacuum line as shown in Figure 2. Stopcock a was provided with a pressure fitting and the ground joint was fitted with springs. (Dow-Corning silicone high-vacuum grease was used in the stopcock and the ground joint, since the apparatus was to be heated in an oven). The tube was evacuated to a pressure of 10^{-5} cm. of Hg, stopcock a was closed, and the assembly was then removed from the vacuum line and placed for a predetermined time in an oven regulated at a desired temperature. After removal from the oven and cooling, the assembly was returned to the vacuum line and the connection above stopcock a evacuated. Then, with a cellosolve-dry ice bath around A to retain the aniline produced, the carbon dioxide was transferred to tube C by cooling the latter in liquid nitrogen. Further purification of the carbon dioxide was effected by distilling it from C at cellosolve-dry ice temperature into either B or D at liquid nitrogen temperature.

In the experiments in which time and temperature conditions for approximately 10 per cent decomposition and for complete decomposition were being determined, the carbon dioxide was frozen in B. This tube was then transferred to another apparatus where the carbon dioxide was slowly swept through a semi-micro combustion type absorption tube filled with ascarite, using carbon dioxide-free hydrogen as sweep gas.

In the experiments where the carbon dioxide was desired for mass spectrometer analysis, it was frozen in tube D. This tube, and a section of the line connecting it to a capillary bore manometer, were previously calibrated so that pressures could be converted into mg. of carbon dioxide. The carbon dioxide was allowed to warm to room temperature and the temperature and manometer reading were noted. The carbon dioxide was then frozen in an evacuated 50 ml. flask containing 5 ml. of frozen, degassed, carbon dioxide-free distilled water. The flask was isolated from the vacuum line and the carbon dioxide and water were allowed to equilibrate overnight at room temperature to ensure that all samples of carbon dioxide had the same oxygen isotopic content. The carbon dioxide was then distilled between cellosolve-dry ice and liquid nitrogen temperatures, dried over phosphorus pentoxide, and then condensed into sample tubes for mass spectrometer analysis.

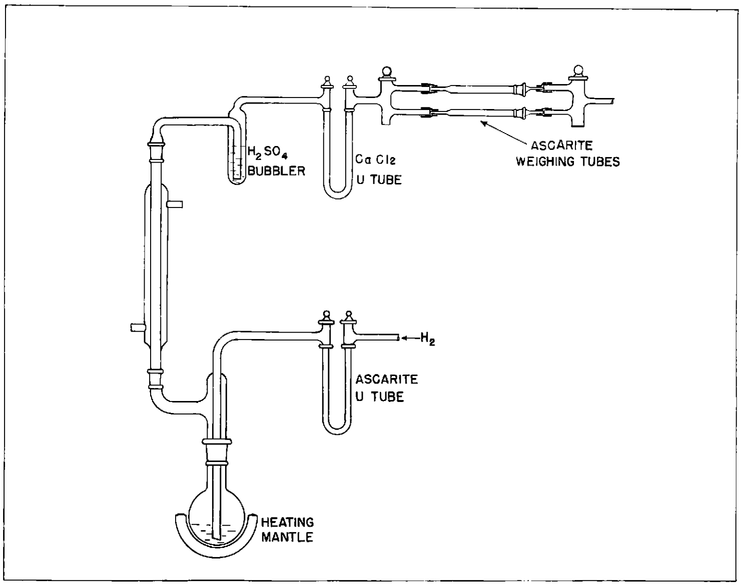


Figure 3. Apparatus for aqueous decarboxylation of anthranilic acid

b. Aqueous Decarboxylation

McMaster and Shriner (44) found that anthranilic acid decarboxylated in boiling water. Under the conditions of their experiments, in which a solution of the acid was allowed to boil in an open flask, the decomposition was found to be first order by these authors.

The apparatus used to investigate the kinetics of the aqueous decarboxylation under the conditions used here is shown schematically in Figure 3. A one gram sample of anthranilic acid was placed in the flask and 70 ml. of carbon dioxide-free water were added. The system was swept with hydrogen until carbon dioxide-free. The flask and contents were then heated to boiling and kept at a gentle boil while a slow stream of hydrogen swept out the carbon dioxide produced. The sulphuric acid trap removed any aniline which passed the condenser, and the acid trap and calcium chloride U-tube removed water. The absorption tubes were weighed alternately after various time intervals to determine the carbon dioxide produced.

For the runs in which the carbon dioxide was required for mass spectrometer analysis, the absorption tubes were replaced by tube B, shown in Figure 2, which was surrounded by liquid nitrogen. Tube B, thus cooled, was shown to be efficient for condensing small quantities of carbon dioxide from a hydrogen stream by slowly generating known quantities of carbon dioxide in the line at the flask position and measuring the amount collected in tube B either in the calibrated volume of the vacuum line or by absorption in ascarite. The carbon dioxide samples collected from the runs for mass spectrometer analyses were measured in the calibrated volume of the vacuum line and then treated in the same way as samples obtained from the thermal decarboxylations. Only samples of carbon dioxide from partial decarboxylations were required and obtained from the aqueous runs for mass spectrometer analyses.

c. Acid-Catalyzed Aqueous Decarboxylation

It was found that the decarboxylation of anthranilic acid was catalyzed by the addition of small amounts of mineral acid. The kinetics of this reaction were studied over a range of mineral acid concentration and a sample of carbon dioxide produced during partial decarboxylation of anthranilic acid in sulphuric acid was analyzed with the mass spectrometer. The methods used were the same as those described for the aqueous experiments except that various concentrations of sulphuric acid from 0.25 N to 3 N were used instead of distilled water.

d. Sodium Anthranilate

An equivalent amount of 0.1 N sodium hydroxide solution was added to a one gram sample of anthranilic acid, and the volume was made up to 70 ml. with distilled water. This solution was placed in the flask used for the other aqueous runs. The apparatus was modified by the addition of a ground-glass Y connection between the condenser and the flask. A separatory funnel was placed in one arm of the Y connection and the condenser in the other arm. After two hours of boiling, the solution was cooled and dilute sulphuric acid was added through the separatory funnel. Sufficient time was allowed for any carbon dioxide produced to be swept through the system and the ascarite tube was then weighed. No increase in weight was found.

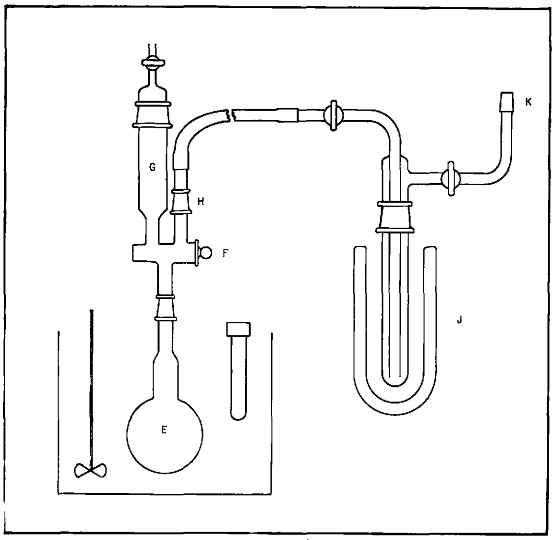


Figure 4. Apparatus for decarboxylation of mesitoic acid

10. The Decarboxylation of Mesitoic Acid-Carboxyl-C^{13},C^{14}

The decarboxylation of mesitoic acid in concentrated solutions of sulphuric acid was recently studied by Schubert (65) for the purpose of determining the reaction mechanism. Bothner-By and Bigeleisen (12) more recently measured the C^{13} isotope effect in the same reaction.

a. Partial Decarboxylation

The apparatus used for decarboxylation of the mesitoic acid is shown in Figures 4 and 2. The 100 ml. reaction flask, E in Figure 4, was fitted with a three-way stopcock, F, to which was attached a delivery funnel, G. Stopcock F was attached to a length of rubber pressure tubing through a ground glass joint, H. The other end of the pressure tubing was attached to a cold trap, J. The trap was attached to the high-vacuum manifold, shown in Figure 2, through the ground glass joint, K, and was placed in the position occupied by tube A. The trap was surrounded by a cellosolve-dry ice bath. During reaction, the reaction flask was held in a Burrell "Wrist Action Shaker", so arranged that the flask could be continuously shaken in a hot water bath. The bath was mechanically stirred and was heated by a Fisher "Automerse" heater. The flask joint and stopcock F were lubricated with Dow-Corning high vacuum silicone grease, since hydrocarbon greases were attacked by the concentrated sulphuric acid used.

A sample of mesitoic acid, weighing approximately 300 mg., was accurately weighed into the reaction flask. The flask, stopcock F, and funnel G were evacuated to 10^{-3} mm. pressure, and stopcock F was closed. During this evacuation, the flask was not placed in the hot water bath because the mesitoic acid tended to sublime. After evacuation, the flask was placed in the bath which was maintained at 60 ± 0.5°C. A 52 ml. sample of 82.2 per cent sulphuric acid was placed in a flask similar to E and was connected to a vacuum pump through a ground glass joint, a length of rubber pressure tubing, and a cold trap. The flask, containing the sulphuric acid, was placed in the hot water bath and was gently shaken while it was carefully evacuated and pumped on for about 10 minutes. If the sulphuric acid was not treated in this way, a small volume of non-condensable gas was produced during the decarboxylation of the mesitoic acid. When the sulphuric acid had reached the bath temperature, the stop-cock attached to the cap on funnel G was opened and the cap was removed. The sulphuric acid was transferred to the funnel and was carefully run into the reaction flask leaving 2 ml. of sulphuric acid in the funnel. The shaker was started and the time noted. The system to the right of stopcock F was evacuated to 10^{-3} mm. and was then isolated from the pumps before stopcock F was opened.

In preliminary dummy runs, liquid nitrogen was placed around tube D, shown in Figure 2. Stopcock F was closed at various time intervals, and the carbon dioxide which had collected in tube D was allowed to expand into the calibrated volume of the vacuum system for measurement. The gas was then re-frozen into D, and stopcock F and the isolating stopcock on the manifold were opened again. More carbon dioxide was allowed to collect and the measurement was repeated. These runs gave the desired information on the reaction rate.

In the runs in which the carbon dioxide produced was analyzed in the mass spectrometer, liquid nitrogen was placed around tube C, shown in

Figure 2. After six minutes shaking time, the shaker was stopped, stopcock F and the stopcock on the outlet side of trap J were closed. The carbon dioxide which had collected in tube C was slowly sublimed into tube D by placing a cellosolve-dry ice bath around C and a liquid nitrogen bath around D. The isolating stopcock on the vacuum manifold between C and D was closed and the carbon dioxide was allowed to expand into the calibrated volume. The manometer reading and temperature were noted and the gas was then re-frozen into D. The carbon dioxide was then sublimed into a 50 ml. equilibration flask which now replaced tube C, using cellosolve-dry ice and liquid nitrogen baths. The evacuated equilibration flask contained 5 ml. of frozen, degassed, carbon dioxide-free water. The carbon dioxide and water were allowed to equilibrate overnight. A 100 ml. flask containing phosphorus pentoxide spread on the bottom, and which had a small inverted L-shaped side arm, was connected to the vacuum manifold and evacuated. The water in the equilibration flask was then frozen using a cellosolve-dry ice bath, and the carbon dioxide was condensed into the side arm of the drying flask by cooling the side arm with liquid nitrogen. The equilibration flask was isolated by turning its stop-cock, the water was allowed to thaw and was then re-frozen. Any carbon dioxide released in this process was then frozen into the drying flask. The stop-cock on the drying flask was closed and the carbon dioxide was allowed to expand. The carbon dioxide was allowed to stand over the phosphorus pentoxide for about one half hour and was then condensed into a sample tube for mass spectrometer analysis.

b. Complete Decarboxylation

Attempts to completely decarboxylate samples of mesitoic acid at 60°C yielded only 89 to 93 per cent of the expected carbon dioxide in dummy runs. Runs made at 99 ± 0.5°C gave more complete decarboxylation, so that this higher temperature was used to obtain carbon dioxide samples corresponding to almost total decarboxylation.

The procedure followed was the same as for the partial decarboxylation runs at 60°C except for the following modifications: A smaller sample of mesitoic acid, approximately 100 mg., was used. The sulphuric acid was not prewarmed to the water bath temperature, which was 99 ± 0.5°C, but only to about 60°C in an auxiliary bath, while being shaken and exhausted. The reaction flask was not placed in the hot water bath until after the sulphuric acid had been added.

The reaction was over 90 per cent complete in thirty minutes and ceased after about three hours, by which time 98 to 99 per cent of the theoretical yield of carbon dioxide had been produced. The carbon dioxide obtained was then purified, equilibrated, and put into sample tubes for mass spectrometer analysis as described for the partial decarboxylation runs.

IV RESULTS AND DISCUSSION

1. Relative Reaction Rate Equation

In a study of the effect which substitution of C^{13} and C^{14} for C^{12} has on the reaction rate of a compound, one may consider the C^{13} compound, or C^{14} compound, and the normal C^{12} compound as two separate substances reacting simultaneously, in the same reaction vessel and under the same conditions, at slightly different rates.

Consider a general unidirectional reaction

$$C + B + D + \ldots\ldots \xrightarrow{k} M + N + \ldots\ldots$$

$$C^1 + B + D + \ldots\ldots \xrightarrow{k^1} M^1 + N + \ldots\ldots$$

where C is the C^{12} compound in which substitution of a heavy carbon is to be studied and C^1 is the C^{13} or C^{14} compound. The rate constants for the two reactions are k and k^1.

Let c and c^1 be the initial concentrations of the C^{12} and C^{13} or C^{14} compounds, respectively, in moles per litre.

Let b, d, etc. be the initial concentrations of the other reactants.

Let x and x^1 be the moles per litre of C and C^1 which have reacted in time t.

Let y, z, etc. be the moles per litre of B, D, etc. which have reacted in time t.

The concentrations of the various species at time t are: $(c - x)$, $(c^1 - x^1)$, $(b - y)$, $(d - z)$, etc. moles per litre.

$$\text{Then,} \quad \frac{dx}{dt} = k(c - x)(b - y)(d - z) \text{ etc.} \quad \ldots\ldots\ldots\ldots (17)$$

$$\text{and} \quad \frac{dx^1}{dt} = k^1(c^1 - x^1)(b - y)(d - z) \text{ etc.} \quad \ldots\ldots\ldots\ldots (18)$$

Dividing equation (17) by equation (18),

$$\frac{dx/dt}{dx^1/dt} = \frac{k(c - x)(b - y)(d - z) \ldots}{k^1(c^1 - x^1)(b - y)(d - z) \ldots}$$

$$= \frac{k(c - x)}{k^1(c^1 - x^1)} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (19)$$

Integrating equation (19),

$$\frac{k}{k^1} = \frac{\ln \frac{c}{(c-x)}}{\ln \frac{c^1}{(c^1-x^1)}} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (20)$$

It is seen that the same equation results, whatever the overall kinetic order of the reaction, provided it is first order with respect to compound C.

Let $c^1/c = a$ and $x^1/x = a^1$, and let $a^1/a = r$

Then $c^1 = ac$, and $x^1 = a^1x = arx$

If the reaction considered is allowed to proceed until $x/c = f$, then substituting cf for x, $arcf$ for x^1, and ac for c^1 in equation (20),

$$\frac{k}{k^1} = \frac{\ln \frac{c}{(c - cf)}}{\ln \frac{ac}{(ac - acrf)}} = \frac{\ln \frac{1}{(1 - f)}}{\ln \frac{1}{(1 - rf)}}$$

$$= \frac{\ln(1 - f)}{\ln(1 - rf)} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (21)$$

As defined, f is not readily determined experimentally. In the ester hydrolyses, the concentration of C^{14} used was extremely small so that $(x + x^1)/(c + c^1)$, which was actually measured, is almost the same as x/c, and f is thus very closely approximated by the value for the fraction of ester hydrolyzed.

In the anthranilic acid and mesitoic acid decarboxylations, the C^{13} concentration, and in the latter acid, the C^{14} concentration, was close to 1 per cent. However, even in these experiments, the uncertainty in the measurement of $(x + x^1)/(c + c^1)$, the fraction of compound reacted, was certainly greater than any error which would be introduced by considering f to be equal to $(x + x^1)/(c + c^1)$, instead of x/c. The ratio k/k^1, as determined by equation (21) is, in any case, not very sensitive to small errors in f, provided f is not much greater than 0.5. In these experiments, f was never greater than 0.2.

The radioactivity measurements give values which are directly proportional to $x^1/(x + x^1)$ and $c^1/(c + c^1)$, and in the ratio

$$\frac{x^1}{(x + x^1)} \Big/ \frac{c}{(c + c^1)},$$

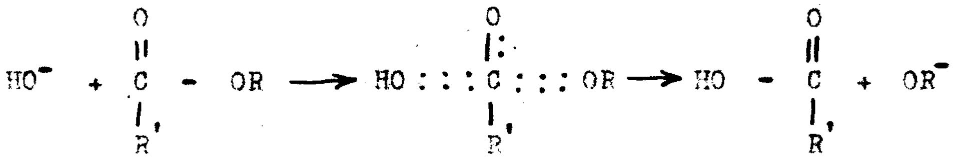
the proportionality constant cancels out. Since $x^1 \ll x$ and $c^1 \ll c$, the errors introduced if $x^1/(x + x^1)$ and $c^1/(c + c^1)$ are considered to be equal to x^1/x and c^1/c, are negligible. Hence r can be considered to be the ratio of the specific activity of a reacted fraction of a sample of compound to the specific activity of the initial starting material, both being expressed as counts per micromole.

The mass spectrometer analyses give values for x^1/x and c^1/c directly, and r is thus the ratio of the respective relative abundances of heavy to light carbon atoms in a reacted fraction and in the initial material.

Equation (21) may thus be used to express the C^{13} or C^{14} isotope effect in terms of relative reaction rates, where *f* is the *fraction* of the compound reacted, and *r* is either the *ratio* of the relative abundances of the isotopes in the reacted fraction and the initial material, or the ratio of the specific activities of the reacted fraction and the initial material.

2. Alkaline Hydrolysis of Ethyl Benzoate-Carboxyl-C^{14}

The alkaline hydrolysis of ethyl benzoate is almost certainly a bimolecular, S_2, replacement reaction. Dewar, in his book "The Electronic Theory of Organic Chemistry" (Oxford University Press, Amen House, London E.C. 4, England, 1949) formulates such hydrolyses as:



$$HO^- + \overset{\overset{O}{\|}}{\underset{\underset{R'}{|}}{C}} - OR \longrightarrow HO:::\overset{\overset{O}{|:}}{\underset{\underset{R'}{|}}{C}}:::OR \longrightarrow HO - \overset{\overset{O}{\|}}{\underset{\underset{R'}{|}}{C}} + OR^-$$

In this particular hydrolysis, R' and R are phenyl and ethyl groups, respectively. In the transition state, the alkoxyl and hydroxyl groups are linked to the central carbon by partial double bonds. Otherwise, the reaction resembles a normal S_2 replacement reaction. The reaction rate is given by the equation $v = k(\text{ester}) \times (OH^-)$. Equation (21) may therefore be used to calculate the C^{14} isotope effect on the reaction rate of the hydrolysis, using the experimentally determined values of the fraction of ester hydrolyzed and the ratio of the specific activities for f and r, respectively.

The analytical data for the alkaline hydrolysis of ethyl benzoate-carboxyl-C^{14} are given in Table I. The two experiments 1 E.B. and 2 E.B. were done about two years apart, and with different labeled ester. The technique used to determine the extent of hydrolysis, imposed by the rapidity of the hydrolysis, could hardly be expected to give very accurate results. The values for percentage hydrolysis may therefore be in error by a considerable amount. Fortunately, the equation for the relative reaction rate ratio is not very sensitive to relatively large errors in the value for the extent of reaction. If the actual extent of reaction were less than the experimental value, the calculated isotope effect would be smaller, and vice versa.

TABLE I

ANALYTICAL DATA - ALKALINE HYDROLYSIS
OF ETHYL BENZOATE-CARBOXYL-C^{14}
(Room Temperature, ~22°C)

Experiment	Hydrolysis Time	Amount of Benzoic Acid Produced (μ moles/ml.)	% Hydrolysis
Total hydrolysis, 1 E.B.	1 week	98.0	100
Partial hydrolysis, 1 E.B.	2 min.	9.9	10.1
Total hydrolysis, 2 E.B.	1 week	35.0	100
Partial hydrolysis, 2 E.B.	1.5 min.	3.4	9.7

The observations and calculations for a typical radioactivity measurement are shown in Table II.

TABLE II

TYPICAL RADIOACTIVITY MEASUREMENT

Tertiary butyl benzoate-carboxyl-C^{14}
Total hydrolysis, alkaline
Volumetric flask No. 83
Titre: 3.27 ml. of 0.0408 N NaOH $\equiv$ 133.6 μ moles/25 ml.
$\equiv$ 0.0651 mg./100 λ $\equiv$ 0.534 μ moles/100 λ

Counting Disc	Sample Volume (λ)	Counting Time (min.)	Scaler (Scale of 64)	Counts per 10 min.
Background		2	3 + 12	1020
Standard		2	122 + 40	
83-1	100	10	125 + 0	8000
83-2	100	10	128 + 22	8214
83-3	100	10	129 + 0	8256
83-4	100	10	131 + 12	8396
Background		2	3 + 5	985
83-5	100	10	129 + 21	8277
83-6	100	10	126 + 0	8064
83-7	100	10	129 + 12	8268
83-1	100	10	127 + 57	8185
83-6	100	10	131 + 23	8407
Standard		2	123 + 12	
Background		2	3 + 7	995

Counting Disc	Counts per 10 min.	Deviation	Deviation^2
83-1 (Respread)	8,185	-77	5,929
83-2	8,214	-48	2,304
83-3	8,256	-6	36
83-4	8,396	+134	17,956
83-5	8,277	+15	225
83-6 (average)	8,236	-26	676
83-7	8,268	+6	36
Total	57,832		27,162
Average	8,262		

Standard deviation of mean value $= \sqrt{\frac{27,162}{7 \times 6}} = \pm 26$

Background 1,000 counts/10 min. (higher than usual)

Counts/μ mole/min. $= \frac{8262 - 1000}{10 \times 0.534} \pm 26 \times \frac{1360}{7262}$

$= 1360 \pm 5$

The C^{14} isotope effect results for alkaline hydrolysis of ethyl benzoate-carboxyl-C^{14} are given in Table III. The two values obtained for k12/k14 are not in particularly good agreement, but do overlap. Bigeleisen and Allen (7) give a formula for the fractional error in the reaction rate ratio as a function of f and r, and the errors in f and r:

$$\frac{[k_1/k_2-1]}{[k_1/k_2-1]} = \frac{[f/(1-f)]\ \delta(1-r) + (1-r)\,\delta[(f/(1-f)^2]}{\ln[1 + (1-r)f/(1-f)]} + \frac{\delta f}{(1-f)\ln(1-f)}$$

The symbol δ refers to an error in the term which follows. As these authors have pointed out, an error of 2 per cent in the relative specific activities leads to a 30 per cent error in the reaction rate ratio. It is unfortunate that the specific activities of C^{14} compounds can not be more accurately determined, but the fact remains that as accuracy of about ± 2 per cent is the best that can be expected at present.

The lower of the two values for the reaction rate ratio found for this ester hydrolysis does not seem to be out of line with the majority of the C^{14} isotope effects which have been measured. The first value found seems to be somewhat high. However, Fry and Calvin's (29) values for the intramolecular C^{14} isotope effect in the decarboxylation of phenyl-malonic acid are 1.097 at 163°C and 1.132 at 72.8°C. The isotope effect at 20° to 25°C would presumably be larger.

TABLE III

C^{14} ISOTOPE EFFECT IN ALKALINE HYDROLYSIS OF ETHYL BENZOATE-CARBOXYL-C^{14}

Experiment	Fraction of Ester Hydrolyzed = f	Concentration of Benzoic Acid (μ moles/100λ)	Counts/100 λ /min.	Counts /μ mole/min.	Ratio of Activities = r	k12/k14
1 E.B.	1.0	0.706	807 ± 4	1141 ± 6	0.878 ± 0.016	1.146 ± 0.017
1 E.B.	1.0	1.405	1611 ± 8	1145 ± 6		
1 E.B.	0.101	0.594	595 ± 3	1002 ± 6		
1 E.B.	0.101	1.185	1192 ± 6	1006 ± 6		
2 E.B.	1.0	0.490	5,326 ± 54	10,870 ± 80	0.903 ± 0.02	1.114 ± 0.021
2 E.B.	0.097	0.491	4,810 ± 47	9,820 ± 95		

3. Acid and Alkaline Hydrolysis of Tertiary Butyl Benzoate-Carboxyl-C^{14}

The mechanism for acid hydrolysis of primary and secondary esters is not quite so clearly defined as is the mechanism for alkaline hydrolysis of these esters. However, all available evidence indicates that acyl oxygen bond rupture occurs when these esters hydrolyze in acid solution. Final proof of this fact was obtained by Datta, Day, and Ingold (22) who found that acid-catalyzed hydrolysis of methyl hydrogen succinate in water enriched in O^{18} gives methanol free from excess O^{18}. Since the acyl oxygen bond rupture is considered to be the rate controlling step in the reaction, the mechanism for acid-catalyzed hydrolysis of primary and secondary esters is basically very similar to the mechanism for alkaline hydrolysis of these esters. Cohen and Schneider (20) have obtained evidence that the mechanism of acid-catalyzed hydrolysis of tertiary butyl benzoate is different, however, and involves alkyl oxygen bond rupture. Alkaline hydrolysis of this ester, on the other hand, was found to be normal and to involve acyl oxygen bond rupture.

The same bond ruptures in both acid-catalyzed and alkaline hydrolysis of an ester such as ethyl benzoate and, in each case, the cleavage appears to be rate controlling. Since a C^{14} isotope effect was found in alkaline hydrolysis of ethyl benzoate-carboxyl-C^{14}, it was reasonable to expect that an isotope effect would be found in acid hydrolysis of the same ester. Acid hydrolysis of ethyl-benzoate-carboxyl-C^{14} was investigated, nevertheless, to make certain that an isotope effect did occur in the reaction, as expected. On the basis of the work done by Cohen and Schneider, it could be expected that no significant C^{14} isotope effect would occur during acid hydrolysis of tertiary butyl benzoate-C^{14}, since the isotopic atoms would not be directly involved in the reaction. On the other hand, a C^{14} isotope effect would be expected to occur in alkaline hydrolysis of the same material, since the isotopic atoms would be directly involved in the reaction.

The results obtained for the C^{14} isotope effects in acid and alkaline hydrolysis of tertiary butyl benzoate-carboxyl-C^{14}, and in acid hydrolysis of ethyl benzoate-carboxyl-C^{14}, were very nearly as expected. The analytical information for the three different hydrolyses is given in Tables IV, V, and VI. The radioactivity measurements and calculated C^{14} isotope effects are given in Tables VII, VIII and IX.

TABLE IV

ANALYTICAL DATA - ACID HYDROLYSIS
OF ETHYL BENZOATE-CARBOXYL-C^{14}
(58.5° ± 0.5°C)

Experiment	Hydrolysis Time	Amount of Benzoic Acid Produced (μ moles/ml.)	% Hydrolysis
Total hydrolysis, 3 E.B.	8 days	34.8	100
Partial hydrolysis, 3 E.B.	13.5 hrs.	3.86	11.1
Total hydrolysis, 4 E.B.	8 days	34.0	100
Partial hydrolysis, 4 E.B.	13.5 hrs.	3.98	11.7

TABLE V

ANALYTICAL DATA - ALKALINE HYDROLYSIS OF TERTIARY BUTYL BENZOATE-CARBOXYL-C^{14}
(Room Temperature, ~ 22°C)

Experiment	Hydrolysis Time	Amount of Benzoic Acid Produced (μ moles/ml.)	% Hydrolysis
Total hydrolysis, 1 T.B.B.	12 days	28.1	100
Partial hydrolysis, 1 T.B.B.	7 hrs.	3.12	11.1
Total hydrolysis, 2 T.B.B.	11 days	28.1	100
Partial hydrolysis, 2 T.B.B.	7 hrs.	2.75	9.8

TABLE VI

ANALYTICAL DATA - ACID HYDROLYSIS OF TERTIARY BUTYL BENZOATE-CARBOXYL-C^{14}
(Room Temperature, ~ 22°C)

Experiment	Hydrolysis Time	Amount of Benzoic Acid Produced (μ moles/ml.)	% Hydrolysis
Total hydrolysis, 3 T.B.B.	13 days (alkaline)	28.1	100
Partial hydrolysis, 3 T.B.B.	28 hrs.	2.50	8.9
Total hydrolysis, 4 T.B.B.	11 days (alkaline)	22.3	100
Partial hydrolysis, 4 T.B.B.	28 hrs.	1.83	8.2

TABLE VII

C^{14} ISOTOPE EFFECT IN ACID HYDROLYSIS OF ETHYL BENZOATE-CARBOXYL-C^{14}

Experiment	Fraction of Ester Hydrolyzed = f	Concentration of Benzoic Acid (μ moles/ml.)	Counts/100 λ /min.	Counts /μ mole/min.	Activities = r	k12/k14
3 E.B.	1.0	0.581	6,221 ± 55	10,710 ± 95	0.938 ± 0.017	1.07 ± 0.018
3 E.B.	0.111	0.642	6,453 ± 61	10,050 ± 95		
4 E.B.	1.0	0.599	6,382 ± 48	10,650 ± 80	0.947 ± 0.017	1.06 ± 0.018
4 E.B.	0.117	0.702	7,079 ± 70	10,100 ± 100		

TABLE VIII

C^{14} ISOTOPE EFFECT IN ALKALINE HYDROLYSIS OF TERTIARY BUTYL BENZOATE-CARBOXYL-C^{14}

Experiment	Fraction of Ester Hydrolyzed = f	Concentration of Benzoic Acid (μ moles/ml.)	Counts/100 λ /min.	Counts /μ mole/min.	Ratio of Activities = r	k12/k14
1 T.B.B.	1.0	0.534	7,262 ± 26	1,360 ± 5	0.938 ± 0.006	1.072 ± 0.007
1 T.B.B.	0.111	0.586	7,461 ± 24	1,275 ± 5		
2 T.B.B.	1.0	0.532	7,320 ± 31	1,378 ± 6	0.909 ± 0.007	1.106 ± 0.008
2 T.B.B.	0.098	0.518	6,481 ± 25	1,252 ± 6		

TABLE IX

C^{14} ISOTOPE EFFECT IN ACID HYDROLYSIS OF TERTIARY BUTYL BENZOATE-CARBOXYL-C^{14}

Experiment	Fraction of Ester Hydrolyxed = f	Concentration of Benzoic Acid (μ moles/ml.)	Counts/100 λ /10 min.	Counts /μ mole/min.
3 T.B.B.	1.0	0.530	7,311 ± 28	1,380 ± 6
3 T.B.B.	0.089	0.473	7,512 ± 29	1,376 ± 6
4 T.B.B.	1.0	0.418	5,678 ± 24	1,361 ± 6
4 T.B.B.	0.082	0.353	4,776 ± 23	1,355 ± 6

The k12/k14 ratios for acid hydrolysis of ethyl benzoate-carboxyl-C^{14} are in fair agreement. The C^{14} isotope effect found in this reaction is only slightly greater than one-half that found in the alkaline hydrolysis of the same ester. Acid hydrolysis is reversible to some extent, however, which may explain part of the difference. Some of the benzoic acid produced during partial hydrolysis might be esterified by the ethyl alcohol in the hydrolysis solution. In the esterification, although it is possible that heavy benzoic acid could have the greater rate constant, it is more probable that light benzoic acid has the greater rate constant. Esterification would thus tend to increase the amount of benzoic acid-carboxyl-C^{14} present in the partially hydrolyzed solution. The apparent C^{14} isotope effect in the hydrolysis would then be smaller than the actual isotope effect. The other factor which tends to make the C^{14} isotope effect smaller in this acid hydrolysis is that the temperature was 58.5°C, compared to room temperature for the alkaline hydrolysis. Isotope effects in unidirectional processes become smaller as the temperature increases. (29, 30, 60).

The agreement for the k12/k14 ratios obtained for alkaline hydrolysis of tertiary butyl benzoate-carboxyl-C^{14} is not good. This is somewhat surprising since the indicated precision of the specific activity measurements is rather better than usual. However, there is no doubt that an appreciable C^{14} isotope effect does occur in the alkaline hydrolysis.

The agreement between the specific activities of the benzoic acid samples obtained from the acid hydrolysis of tertiary butyl benzoate-carboxyl-C^{14} experiments is quite good. From these specific activities, it is obvious that no appreciable C^{14} isotope effect occurs in acid hydrolysis of the tertiary butyl ester. It is conceivable that the esterification isotope effect discussed above could operate in this reaction to such an extent as to almost completely nullify the hydrolysis isotope effect. This possibility is not considered very likely, however, since a significant isotope effect was observed in the acid-catalyzed hydrolysis of ethyl benzoate-carboxyl-C^{14} when it was hydrolyzed to approximately the same extent as the tertiary butyl ester.

The C^{14} isotope effects found in the acid and alkaline hydrolysis of the two esters, taken together, are substantial evidence that the mechanism for acid hydrolysis of tertiary butyl benzoate involves an alkyl oxygen bond cleavage as suggested by Cohen and Schneider. These results may also be considered as evidence for the concept that significant isotope effects occur when isotopic molecules react only if isotopic atoms are directly involved in the reaction.

4. The Decarboxylation of Anthranilic Acid

a. Thermal Decarboxylation

Some information on the rate of decarboxylation at three different temperatures was obtained and is shown in Table X. The results agree reasonably well with those of Pawlewski (53). Although the values lack precision, they indicate that the reaction is first order as shown in Figure 5, where log of per cent anthranilic acid remaining is plotted against time. Complete decarboxylation was never obtained at the temperatures studied. Decarboxylation does not appear to occur at temperatures below the melting point of the acid as is seen from Run 17.

b. Aqueous Decarboxylation

The results of a study of the kinetics of the aqueous decarboxylation are given in Table XI. A plot of log of per cent anthranilic acid remaining against time, given in Figure 6, shows that the reaction is first order initially. The reaction rate then decreases too rapidly for the reaction to remain first order. In view of the results of the acid catalyzed and base inhibited runs done later, it is obvious that the build up of aniline in the reaction flask inhibits the decarboxylation. Aniline build up probably accounts for the incompleteness of thermal decarboxylation also. The rate constant, calculated from the straight line portion of the curve, is close to that reported by McMaster and Shriner (44).

c. Aqueous Acid-Catalyzed Decarboxylation

The results of the acid catalyzed decarboxylations are given in Table XII. The reaction, in each case, is first order up to the point where the experiment was stopped. The results for 1N, 2N and 3N sulphuric acid solutions are plotted in Figure 6. The values for 0.25, 0.5 and 0.75 N lie so close to the values for 1 N that they have not been shown in Figure 6.

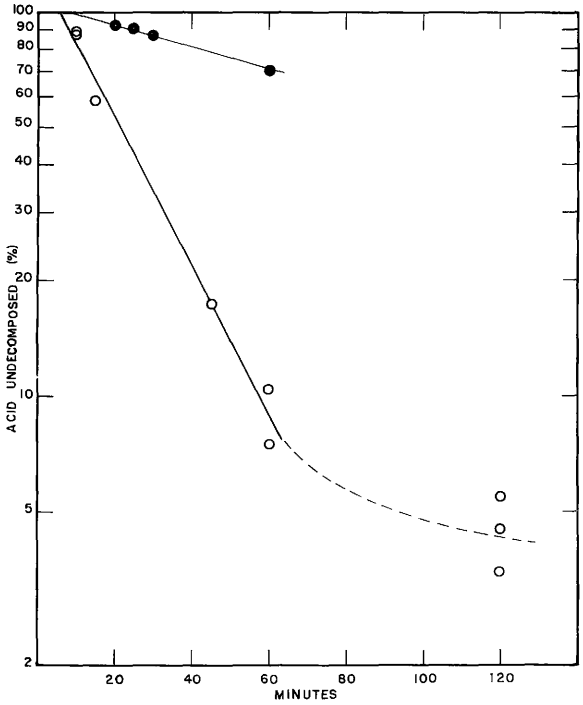


Figure 5. Thermal decarboxylation of anthranilic acid

● 156 ± 4°C
○ 197 ± 4°C

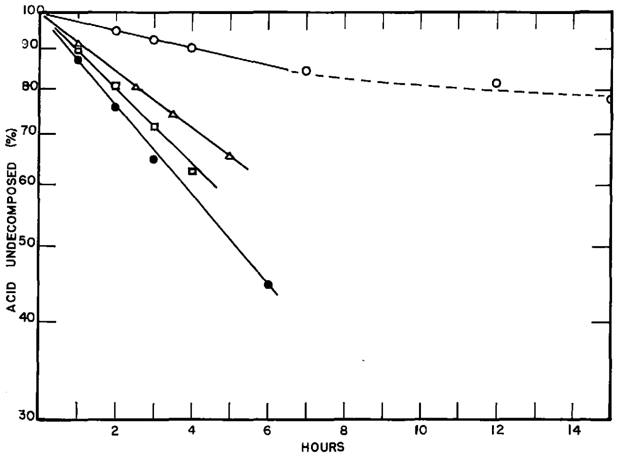


Figure 6. Aqueous and acid-catalyzed decarboxylation of anthranilic acid

- ○ No mineral acid added
- ● 1 N sulphuric acid
- □ 2 N sulphuric acid
- △ 3 N sulphuric acid

TABLE X

THERMAL DECARBOXYLATION OF ANTHRANILIC ACID

Run	Temperature °C	Time in Minutes	% Decomposition
17	140 ± 4	90	0
32	156 ± 4	20	7.0
33		25	10.0
31		30	12.3
30		60	22.9
26	197 ± 3	10	10.8
22		10	13.0
14		15	41.5
12		45	82.2
7		60	89.5
8		60	92.5
9		120	96.5
10		120	94.5
11		150	95.5
Pawlewski	150	60	23.9
"	160	60	47.7
"	190	60	88.3
"	200	60	96.2

TABLE XI

AQUEOUS DECARBOXYLATION OF ANTHRANILIC ACID

		Rate Constant in hours^{-1}	
Time in hours	% Decomposition	Present Work	McMaster & Shriner
2	5.2	0.0268	0.029
3	7.3	0.0257	0.025
4	7.6	0.0250	0.031
7	15.4	0.0240	0.025
12	18.1	0.0165	
15	22.7	0.0172	

d. Mass Spectrometer Analyses

The relative abundance of C^{12} and C^{13} in the carboxyl group of the anthranilic acid was determined from mass spectrometer analyses of the CO_2 obtained from two thermal decarboxylations which were 96 and 98 per cent complete, respectively. If an isotope effect occurred, this value would leave some doubt as to the actual relative abundance of C^{13} and C^{12} in the carboxyl group, because of the incomplete decarboxylation. This uncertainty would lead to a small error in the calculation of the relative reaction rates of the C^{12} and C^{13}-carboxyl anthranilic acids. This difficulty did not arise, however, since no significant isotope effect was observed in the reaction. Samples of gas from partial thermal, partial aqueous, and partial acid-catalyzed decarboxylations were analyzed to determine the C^{13} isotope effect. The mass spectrometer results are given in Table XIII. Since there is a probable error of about 0.1 per cent in the individual mass spectrometer analyses, the maximum difference of 0.5 per cent observed in the C^{13}/C^{12} ratios indicates that any isotope effect in this reaction is an order of magnitude smaller than the effect found in all decarboxylations so far reported, except the decarboxylation of barium adipate (13).

TABLE XII

ACID CATALYZED AQUEOUS DECARBOXYLATION OF ANTHRANILIC ACID

Sulphuric Acid Normality	% Decomposition at time 1 hr.	2 hr.	2.5 hr.	3 hr.	3.5 hr.	4 hr.	4.5 hr.	5 hr.	6 hr.	Rate Constant in $hrs.^{-1} x 10^2$
0.25	12.32	22.62			37.34		45.76		55.13	13.5
0.50	12.21	23.42			35.16		45.54			13.7
0.75	13.81	25.41		35.05		43.03		49.85	56.46	14.1
1.0	12.93	24.27		35.10					55.10	13.9
2.0	10.15	19.62		28.63		37.63				11.6
3.0	8.45		19.82		25.80			34.53		8.5

TABLE XIII

C^{13} ISOTOPE EFFECT IN THE DECARBOXYLATION OF ANTHRANILIC ACID

Expt. No.	Decarboxylation Method	Temp. °C	Wt. Anthranilic Acid (g.)	Wt. CO_2 (mg.)	% Decarboxylation	$C^{13}O_2/C^{12}O_2 \times 10^4$*
1A	Thermal	197 ± 4	0.05549	17.1	96	116.56 ± 0.05
3A	"	197 ± 4	0.05000	15.7	98	116.33 ± 0.05
5A	"	156 ± 4	0.15655	34.6	69	116.65 ± 0.05
2A	"	156 ± 4	0.15976	6.6	13	117.14 ± 0.05
6A	"	156 ± 4	0.15115	4.8	10	116.26 ± 0.06
7A	Aqueous	100	0.50141	16.2	10	116.25 ± 0.06
8A	Acid-Catalyzed Aqueous	100	0.50984	16.4	10	115.71 ± 0.04
Standard						120.56

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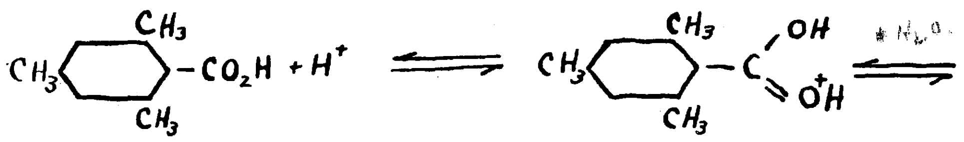
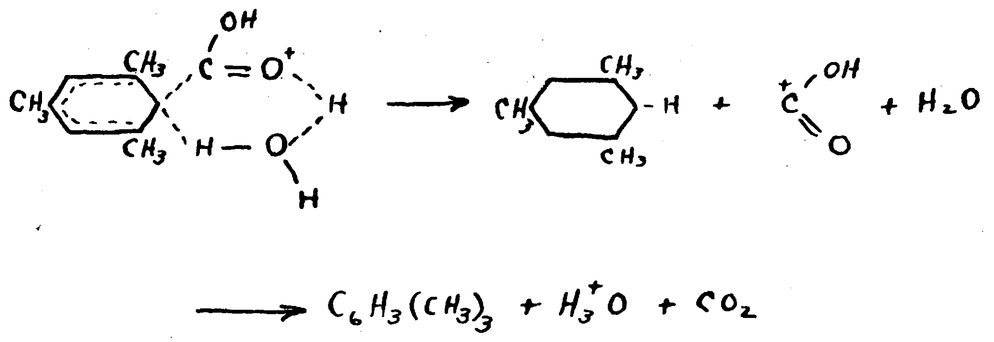
*Precision expressed in term of the standard deviation of the mean

e. Discussion of the Mechanism of Anthranilic Acid Decarboxylation

From the discussion of the theory of isotope effects in unidirectional reactions, it will be appreciated that if a certain bond rupture is the rate determining step in a reaction and if isotopic atoms are directly involved in the bond rupturing, then an isotope effect is expected to occur in the reaction. On this basis, when an acid decarboxylates by a unimolecular mechanism, there should be an isotope effect. For example, an isotope effect has been observed in the decarboxylation of trichloracetic acid (7) which is known to decompose by a unimolecular process. The fact that there is no observed isotope effect in the decarboxylation of anthranilic acid is evidence that the mechanism is not unimolecular.

In 1948, Schenkel and Schenkel-Rudin (63) suggested that some organic acids are decarboxylated by a bimolecular electrophilic substitution mechanism (S_E2). Since then, several examples of S_E2 mechanisms have been found (17, 37, 38). The acid catalysis and base inhibition observed in the decarboxylation of anthranilic acid indicate that the reaction requires hydrogen ions and is bimolecular. However, an isotope effect has been observed in the decomposition of mesitoic acid (12) which has been shown to decompose by a bimolecular process (65). In the mesitoic decarboxylation, the carbon-carbon bond cleavage must be the rate controlling step to account for the isotope effect. The mechanism proposed by Schubert (65),





does satisfy this condition. It is assumed that the formation of the protonated acid, or the solvated ion, is reversible, with a low activation energy, and that the carbon-carbon bond cleavage is rate controlling.

For the anthranilic acid decarboxylations, two possible points of attack by the proton should be considered. The attack may be on a carboxyl oxygen, as proposed for mesitoic acid, or the attack may be on the α carbon as has been suggested by Brown and co-workers for the decarboxylation of o-hydroxybenzoic acid (17). Also, several species of anthranilic acid must be considered. Bjerrum (11) has shown that un-ionized $NH_2C_6H_4CO_2H$ molecules exist in solution to a considerable extent as well as the zwitterions $NH_3^+C_6H_4CO_2^-$. Two ionic forms, $NH_3^+C_6H_4CO_2H$ and $NH_2C_6H_4CO_2^-$, also exist, their amounts depending on the hydrogen ion concentration.

There are four equilibria, mutually dependent:

(i) $NH_2 \cdot R \cdot CO_2H \rightleftharpoons NH_2 \cdot R \cdot CO_2^- + H^+$

(ii) $NH_3^+ \cdot R \cdot CO_2H \rightleftharpoons NH_2 \cdot R \cdot CO_2H + H^+$

(iii) $NH_3^+ \cdot R \cdot CO_2H \rightleftharpoons NH_3^+ \cdot R \cdot CO_2^- + H^+$

(iv) $NH_2 \cdot RCO_2 \cdot H \rightleftharpoons NH_3^+RCO_2^-$

(where $R \equiv C_6H_4$)

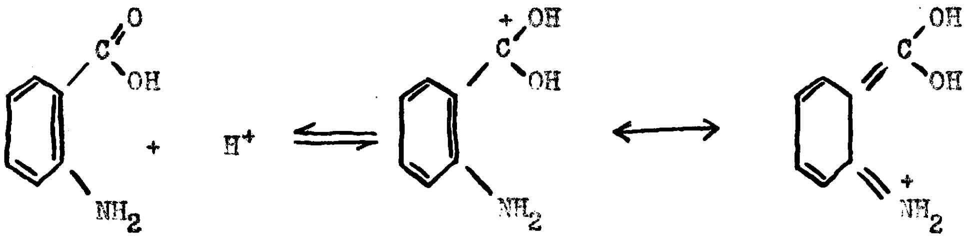
If we use square brackets to indicate concentrations, then $[NH_2 \cdot R \cdot CO_2^-] \times [H^+] = K_1 [NH_2 \cdot R \cdot CO_2H]$. Because $NH_2C_6H_4CO_2H$ is a weak acid, $[NH_2 \cdot R \cdot CO_2H]$ will be very nearly constant, and $[NH_2RCO_2^-] \times [H^+] \cong K_1'$. The decarboxylation rate will be given by the expression, rate $= K [H^+]$ [organic acid species]. Thus, if the ion $NH_2RCO_2^-$ is the reactant, the reaction rate should remain practically constant as the mineral acid concentration is changed. Since the rate increases as the sulphuric acid concentration increases up to about one normal, this species is not likely the reactant. From (ii), $[NH_3^+RCO_2H] \cong K_2' [H^+]$. The reaction rate should thus vary almost directly as the square of the sulphuric acid concentration, and certainly never decrease, if $NH_3^+RCO_2H$ is the reactant. Since the reaction rate decreases when the sulphuric acid concentration increases above 1 N, this species is also almost certainly eliminated as the reactant.

With either the neutral molecule or the zwitterion as reactant, the acid-catalyzed runs are explicable. At low mineral acid concentrations, the increase in hydrogen ion concentration, caused by an addition of mineral acid, is relatively greater than the decrease in either zwitterion or neutral molecule concentration resulting from the addition of the mineral acid. The reaction rate is thus increased by small additions of mineral acid. However, when the hydrogen ion concentration approaches that of the anthranilic acid, reaction (iii) becomes important. $NH_3^+ \cdot R \cdot CO_2H$ is a relatively strong acid due to the inductive effect of the NH_3^+ group. The concentration of $\overset{+}{NH_3} \cdot R \cdot CO_2H$, therefore, will become appreciable only at high hydrogen ion concentrations, where the concentration of the zwitterion $NH_3^+ \cdot R \cdot CO_2^-$, and consequently of the $NH_2 \cdot R \cdot CO_2H$ in equilibrium with it, are both decreased by reaction (iii). At the same time, hydrogen ions are withdrawn from the solution by reaction (iii). It is thus quite conceivable that due to removal of $NH_3^+ \cdot R \cdot CO_2^-$ and $NH_2 \cdot R \cdot CO_2H$ and a corresponding number of hydrogen ions by the same reaction, the reaction rate could decrease with increasing mineral acid addition at some high mineral acid concentration. Hence the reaction rate first increases, and then decreases, as the sulphuric acid concentration is increased. The acid-catalyzed runs are of no help, however, in deciding between the neutral molecule and the zwitterion, since the ratio of their concentrations should be practically insensitive to hydrogen ion concentration.

The possible reaction mechanisms to be considered then, are: (a) proton attack on the oxygen of the neutral molecule, (b) proton attack on the α carbon of the neutral molecule, (c) proton attack on the oxygen of the zwitterion, (d) proton attack on the α carbon of the zwitterion. Obviously, single proton attack on the oxygen of the zwitterion would not lead to decarboxylation, so path (c) is eliminated.

It is difficult to decide in favour of (a), (b), or (d), as the detailed mechanism. If the reaction goes by mechanism (a) or (b), involving the neutral molecule, one might expect p-aminobenzoic acid to react faster than anthranilic, since the ratio of neutral molecules to zwitterions is greater in p-aminobenzoic than in anthranilic acid solutions (11). However, the rate for p-aminobenzoic is only about one half the rate for anthranilic acid (44). Thus, on this basis, (d) would seem to be the preferred mechanism.

In an attack by a proton on the oxygen, mechanism (a),



the bond between the ring carbon and carboxyl carbon would tend to increase in double bond character, and hence in bond strength, through the electromeric effect of the o-amino group as shown. A high activation energy should then be required for the carbon-carbon bond rupture and the cleavage could reasonably be expected to be rate controlling. Mechanism (a) would therefore be likely to show an isotope effect. In the mesitoic acid case, steric hindrance tends to prevent the planar configuration necessary for resonance interaction of the protonated carboxyl group and the ring, thus lowering the activation energy for bond rupture and favouring this mechanism. But even there, the activation energy must be sufficiently high so that the carbon-carbon bond rupture is rate controlling and an isotope effect results. Thus mechanism (a) is discounted because no isotope effect is obtained with anthranilic acid.

In the neutral molecule, there is a high electron density available at the α carbon because of the mesomeric effect of the ortho amino group. This is not true in the zwitterion where the inductive effects of the NH_3^+ and CO_2^- groups probably balance each other fairly closely, and no mesomeric effect is present. Thus the attack of a proton on the α carbon of the zwitterion, mechanism (d), should require a higher activation energy than a similar attack on the neutral molecule. It is known that many acids decarboxylate as zwitterions by a unimolecular process (16, 25, 62, 64). The activation energy for carbon-carbon bond rupture in the zwitterion is thus normally less than that required in the neutral molecule. Mechanism (b) would be much more likely to show an isotope effect than (d) on this basis, since (b) would require a lower activation energy for proton attack and a higher activation energy for carbon-carbon bond rupture than (d).

If it is postulated that the mechanism for the decarboxylation is (d), that is, a frontside attack of a proton on the α carbon of the zwitterion, requiring a high activation energy and being rate controlling, followed by rapid carbon-carbon bond rupture requiring a lower activation energy, the lack of an isotope effect is understandable. Such a two step mechanism is more complex than a normal bimolecular (S_E2) mechanism. It envisions a hypothetical carbonium ion which is stable relative to the activated state through which it passed in its formation, and although unstable to zwitterion formation, relatively more unstable towards decarboxylation. The carbonium ion can be thought of as being in a shallow energy valley between two energy peaks, the higher representing the activation energy for formation of the carbonium ion, the lower representing the activation energy for decarboxylation. No significant isotope effect, as far as decarboxylation is concerned, should occur in the proton attack on the α carbon because the dependence of the activation energy for this step on the mass of the carboxyl carbon can only be very slight. It follows that if there is no isotope effect in the rate controlling step, there will be no isotope effect in the overall reaction.

Thus, while mechanisms (a) and (b) cannot be completely excluded, mechanism (d), which best explains the lack of isotope effect, is considered as being most probable. At the very least, one concludes from the experimental evidence that the decarboxylation is bimolecular, with the attack of a proton somehow being the rate controlling step.

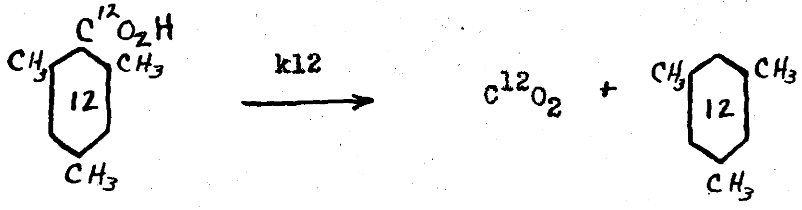
Although the mechanism proposed might appear to apply only to the acid-catalyzed reaction, it is equally applicable to the uncatalyzed aqueous and the thermal decarboxylations. In solution, with no added mineral acid, the anthranilic acid itself, by ionization, supplies the protons for the reaction. Thermal decarboxylation only occurs when the anthranilic acid is in the molten state where again the acid can supply the necessary protons.

5. The Decarboxylation of Mesitoic Acid-Carboxyl-C^{13}, C^{14}

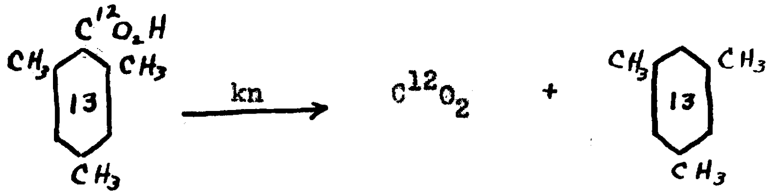
The mesitoic acid is labeled with C^{14} in the carboxyl position, only. The C^{13}, however, occurs in all positions in the natural abundance of about 1 per cent.

The reactions which occur when mesitoic acid-carboxyl-C^{13}, C^{14} is decarboxylated may be represented as:

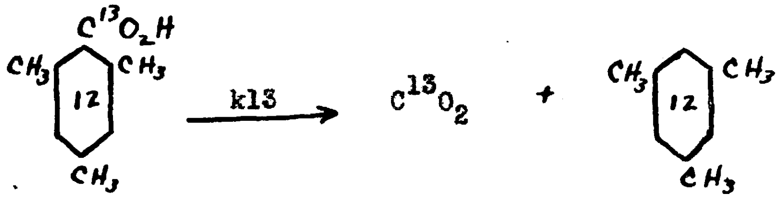
(i)



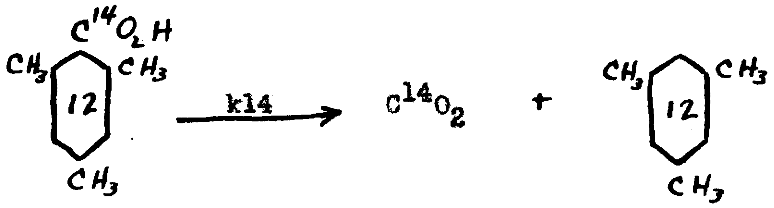
(ii)



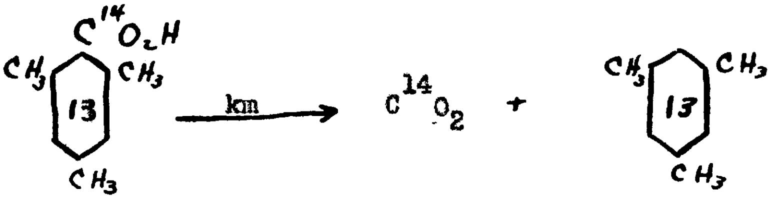
(iii)



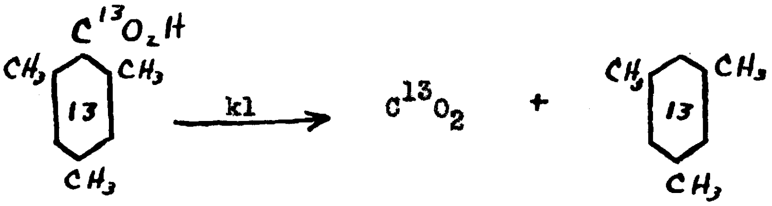
(iv)



(v)



(vi)



The numbers inside the benzene rings refer to the composition of the mesityl group. The number 12 indicates that all carbon atoms in the mesityl group are C^{12} and the number 13 means that one of the carbon atoms is C^{13}. Since there are nine carbon atoms in the mesityl group, equations (ii), (v), and (vi) each represent nine similar equations. Of these twenty-seven equations, only the three which have the C^{13} in the ring and directly attached to the carboxyl group will be subject to any appreciable isotope effect. The fraction of the molecules which will have a C^{13}-carboxyl carbon attached to a C^{13} carbon in the ring, or a C^{14}-carboxyl carbon attached to a C^{13} carbon in the ring, will be of the order of one ten-thousandth, so that reactions (v) and (vi) can be ignored. The molecules which have a C^{13} carbon in the ring attached to a C^{12}-carboxyl carbon which react according to equation (ii), have a reaction rate approximating k13. Since this reaction produces $C^{12}O_2$, the major product of the mixed reactions, the isotope effect changes the $C^{12}O_2$ concentration so slightly that the reaction may be neglected. The neglect of equation (ii) will result in making the observed value for the k12/k13 ratio only very slightly smaller than it actually is. The reactions which are of interest, then, are (i), (iii) and (iv), for which the rate constants are k12, k13, and k14.

The analytical results for the mesitoic acid-carboxyl-C^{13},C^{14} decarboxylation experiments are given in Table XIV. A typical mass spectrogram pair obtained in a mass spectrometric analysis of the carbon dioxide produced by a decarboxylation is shown in Figure 7. The measurements and calculations for this particular spectrogram pair are given in Table XV.

The C^{13}/C^{12} and C^{14}/C^{12} ratios in the various samples of carbon dioxide, which were obtained, are given in Table XVI. These ratios were calculated from the measured $C^{13}O_2/C^{12}O_2$ and $C^{14}O_2/C^{12}O_2$ ratios as described in the experimental section on mass spectrometry. The reaction rate ratios, k12/k13 and k12/k14 were calculated using equation

(21), $k/k^1 = \ln(1-f)/\ln(1-rf)$, and are given in Table XVII.

TABLE XIV

DECARBOXYLATION OF MESITOIC ACID

Expt. No	Temp. °C	Wt. Mesitoic Acid	Wt. CO_2	% Decarboxylation
14 M	60 ± 0.5	0.3198	0.0118	13.7
15 M	60 ± 0.5	0.2947	0.0139	17.5
16 M	99 ± 0.5	0.0925	0.0246	99
17 M	99 ± 0.5	0.0991	0.0263	99

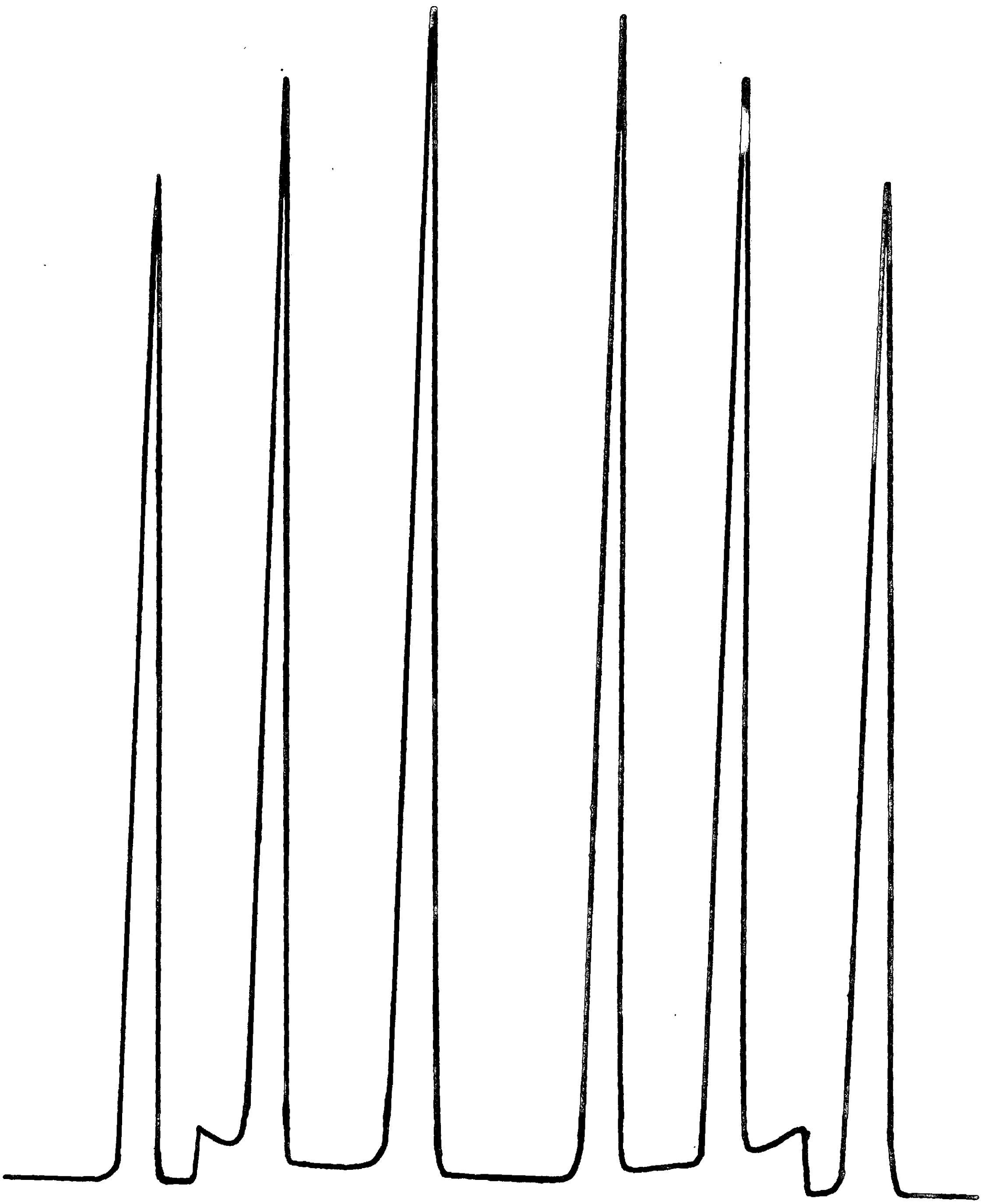


Figure 7. Typical mass spectrogram pair

TABLE XV

MEASUREMENTS AND CALCULATIONS FOR TYPICAL MASS SPECTROGRAM PAIR

(Sample 16 e, Mesitoic Acid-Carboxyl-C^{13},C^{14}, Complete Decarboxylation at 100°C)

Pair No.	Mass	Peak Heights A	Peak Heights B	Average Peak Height	Shunt Factor	Relative Mass Abundance	$C^{13}O_2/C^{12}O_2$ x 10^4	$C^{14}O_2/C^{12}O_2$ x 10^4
7	44	15.32	15.23	15.28	290.4	4437.31		
	45	16.31	16.34	16.33	3.251	53.0888	119.64	
	46	17.51	17.58	17.55	3.251	57.0551		128.58

TABLE XVI

C^{13} AND C^{14} ISOTOPE EFFECTS IN THE DECARBOXYLATION OF MESITOIC ACID

Expt. No.	Temp. °C	% Decarboxylation	$C^{13}O_2/C^{12}O_2$[*] x 10^4	$C^{14}O_2/C^{12}O_2$[*] x 10^4	C^{13}/C^{12} x 10^4	C^{14}/C^{12} x 10^4
16 M	99±0.5	99	119.66±0.05	128.70±0.04	111.64	86.12
17 M	99±0.5	99	120.29±0.04	128.45±0.05	112.27	85.87
Average					111.96	86.00
14 M	60±0.5	13.7	116.35±0.05	120.74±0.08	108.33	78.16
15 M	60±0.5	17.5	115.99±0.06	121.81±0.07	107.97	79.23

Standard CO_2 $C^{13}/C^{12} = 112.54 \times 10^{-4}$

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[*] Precision expressed in terms of the standard deviation of the mean.

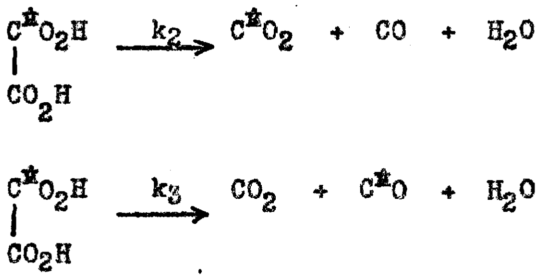
TABLE XVII

DECARBOXYLATION OF MESITOIC ACID - REACTION RATE RATIOS

Expt. No	k12/k13	k12/k14
14 M	1.037 ± 0.001	1.101 ± 0.004
15 M	1.039 ± 0.001	1.094 ± 0.004
Average	1.038	1.101
Bigeleisen and Bothner-By	1.037 ± 0.003	

The ratio obtained for k12/k13 is in good agreement with the value obtained for this ratio by Bigeleisen and Bothner-By (12). The significant observation is that the C^{14} isotope effect is considerably greater than twice the C^{13} isotope effect, whereas present theory predicts that the C^{14} effect should be just twice the C^{13} effect. Using the standard formula, $(A \pm a)/(B \pm b) = A/B \pm \sqrt{a^2/A^2 + b^2/B^2}$, the ratio of the C^{14} and C^{13} isotope effects found in this work is 2.56 ± 0.09.

The only other reaction in which the C^{13} and C^{14} isotope effects have been determined, using the same samples of product for both C^{13} and C^{14} analyses, is the decarboxylation of oxalic acid. Fry and Calvin (30) determined the ratio k_2/k_3 in the reactions



$$\begin{array}{l} C^*O_2H \\ | \\ CO_2H \end{array} \xrightarrow{k_2} C^*O_2 + CO + H_2O$$

$$\begin{array}{l} C^*O_2H \\ | \\ CO_2H \end{array} \xrightarrow{k_3} CO_2 + C^*O + H_2O$$

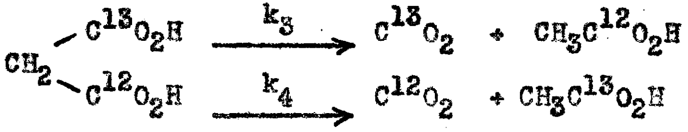
where C^* indicates C^{13} or C^{14}. For C^{13}, $100(k_2/k_3-1)$ was found to be 2.7 ± 0.22 at 103°C and 3.25 ± 0.15 at 80.1°C, from mass spectrometer analyses. For C^{14}, $100\ (k_2/k_3-1)$ was found to be 5.5 ± 0.30 at 103°C and 6.7 ± 0.35 at 80.1°C, from radioactivity measurements, using an ionization chamber and a vibrating reed electrometer. Their C^{13} isotope effect value is somewhat smaller than that obtained by Lindsay, McElcheran and Thode (40) which was 3.2 at 100°, when expressed in the same form as Fry and Calvin's results. The C^{14} isotope effect is seen to be very close to twice the C^{13} isotope effect, as theory predicts. Fry and Calvin considered that both the C^{13} and C^{14} isotope effects found were larger than theory would predict.

The C^{14} isotope effect found in the mesitoic acid decarboxylation appears to be at variance both with theory and with the C^{14} isotope effect found in the oxalic acid decarboxylation by Fry and Calvin. No reasonable explanation for this apparent discrepancy has been found as yet. It might be noted, however, that the C^{13} and C^{14} isotope effects measured in the oxalic acid decomposition are intramolecular effects, whereas the isotope effects measured in the mesitoic acid decarboxylation are intermolecular. The malonic acid decomposition results obtained by Bigeleisen and Friedman (8) and by Lindsay, Bourns, and Thode (39) agreed with theory for the intramolecular C^{13} isotope effect but disagreed with theory for the intermolecular C^{13} isotope effect.

6. General Discussion

About one half of the work presented in this thesis is concerned with the use of C^{13} and C^{14} isotope effect measurements in the elucidation of reaction mechanisms. Information as to which bond ruptures in acid hydrolysis of tertiary butyl benzoate could presumably also be obtained by the use of O^{18}, in experiments similar to those of Datta, Day, and Ingold (22). If the alkoxyl oxygen of the tertiary ester were labeled with O^{18}, then it would be expected that the benzoic acid produced by hydrolysis would contain the O^{18} label, whereas the tertiary butyl alcohol produced would not. The use of the C^{13} isotope effect measurements in the determination of the mechanism by which anthranilic acid decarboxylates is rather more unique. It is difficult to conceive of any other experimentally obtainable evidence which would indicate so clearly that the carbon-carbon bond rupture is not rate controlling in this reaction. From the number of reactions studied to date, in which the measurement of isotope effects has been useful in the elucidation of the reaction mechanism, it seems likely that this technique will prove to be of use in many future mechanism studies. It would be quite useful for this purpose to have more experimental values for isotope effects, particularly values for isotope effects in bond formation reactions.

The other half of the work presented in this thesis is concerned with the measurement of C^{13} and C^{14} isotope effects, per se, and an attempt to obtain an accurate value for a C^{14} isotope effect which could be used to test the Bigeleisen theoretical equation. Actually, the only good experimental evidence for the correctness of the Bigeleisen equation is the value which has been obtained for the intramolecular C^{13} isotope effect in the decarboxylation of malonic acid. The ratio of the two rate constants, k_4/k_3, for the reactions



$$CH_2\begin{cases} C^{13}O_2H \xrightarrow{k_3} C^{13}O_2 + CH_3C^{12}O_2H \\ C^{12}O_2H \xrightarrow{k_4} C^{12}O_2 + CH_3C^{13}O_2H \end{cases}$$

is given by Bigeleisen as:

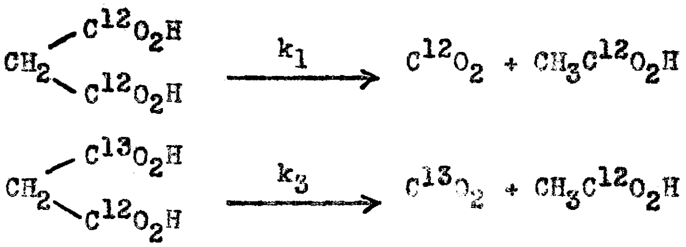
$$\frac{k_4}{k_3} = \left(\frac{m_3^{*}}{m_4^{*}}\right)^{1/2} \left[1 + \sum^{3n-6} G(u_i)\,\Delta u_i - \sum^{3n'-6} G(u_i^{\ddagger})\,\Delta u_i^{\ddagger}\right]$$

For these two reactions, since the substrate molecules are identical, all the Δu_i's are zero and $\sum^{3n-6} G(u_i)\,\Delta u_i = 0$. Bigeleisen then assumes that $\sum^{3n'-6} G(u_i^{\ddagger})\,\Delta u_i^{\ddagger}$ is negligible, which is to say that the activated states for the two reactions are practically identical, and that both carboxyl carbon atoms are essentially free in the activated state. Hence, $\frac{k_4}{k_3} = \left(\frac{m_3^{*}}{m_4^{*}}\right)^{1/2}$. The experimental value for k_4/k_3 is in accord with this theoretical prediction when m_3^{*} and m_4^{*} are taken as the reduced mass of two C^{12} atoms, and of a C^{12} and a C^{13} atom, respectively. Theory gives $k_4/k_3 = 1.0198$, at all temperatures, and the experimental value is 1.02, with no apparent temperature dependence. If it is not assumed that the carboxyl carbons are essentially free in the activated state, then the factor $1 - \sum^{3n'-6} G(u_i^{\ddagger})\,\Delta u_i^{\ddagger}$ may not be considered as being unity, but will be greater than unity. (It should be noted that Δu_i, as defined, is normally positive, but in calculating the intramolecular isotope effect in the malonic acid decarboxylation, it is negative). If the carboxyl carbons are not assumed to be essentially free, then any calculated isotope effect will be larger than the experimental value, since $1 - \sum^{3n'-6} G(u_i^{\ddagger})\,\Delta u_i^{\ddagger}$

will have a value greater than one.

The reduced mass ratio in the equation for the ratio of the reaction rates originates from the factor $(kT/2\pi m^{\ddagger})^{1/2}$ in the Eyring absolute reaction rate theory. This quantity is the average rate of passage of activated complexes over the energy barrier along the coordinate of decomposition. Thus the intramolecular isotope effect in the decarboxylation of malonic acid is all due, according to theory, to the difference in rate of passage of activated complexes over the energy barrier.

Theory and experiment should now be compared for the intermolecular isotope effect. The ratio of the two rate constants, k_1/k_3, for the reactions



is given by Bigeleisen as:

$$\frac{k_1}{2k_3} = \left(\frac{m_3^{\ddagger}}{m_1^{\ddagger}}\right)^{1/2} \left[1 + \sum^{3n-6} G(u_i)\Delta u_i - \sum^{3n'-6} G(u_i^{\ddagger})\Delta u_i^{\ddagger}\right]$$

The largest isotope effect will occur when $\sum^{3n'-6} G(u_i^{\ddagger})\Delta u_i^{\ddagger}$ is zero. Since it has already been assumed that the carboxyl carbons are essentially free in the activated state for the intramolecular reaction, $\sum^{3n'-6} G(u_i^{\ddagger})\Delta u_i^{\ddagger}$ can be assumed to be negligible here, also. Bigeleisen calculated that the upper limit for the ratio $k_1/2\,k_3$ is 1.021, but obtained the experimental value 1.035 for the same ratio. The calculation of $\sum^{3n-6} G(u_i)\Delta u_i$, would not be expected to be in error by an order of magnitude, yet an error of this size must be assumed to reconcile theory with experiment. If $\sum^{3n'-6} G(u_i^{\ddagger})\Delta u_i^{\ddagger}$ is not taken as zero, the agreement between theory and experiment is worse.

The Bigeleisen theory predicts that the C^{14} isotope effect in any reaction will be very nearly twice the C^{13} isotope effect. Many of the C^{14} isotope effects, which have been observed, have been considerably larger than expected on this basis, and all but a very few C^{14} isotope effects have been roughly of the same magnitude. Since errors of 30 per cent, or greater, are easily possible when radioactivity measurements are used to determine the isotope effect, it is difficult to condemn the Bigeleisen theory on the basis of such measurements. The measurement of the C^{14} isotope effect in the decarboxylation of mesitoic acid, using mass spectrometric measurements, is more certain evidence that something is wrong with the Bigeleisen equation, however.

It is difficult to see where the equation might be in error. Even if calculations of the $\sum^{3n-6} G(u_i)\Delta u_i$ factors *are* in error in any example, the C^{14} isotope effect should still be twice the corresponding C^{13} isotope effect. In developing the theoretical equation, Bigeleisen assumes that the transmission coefficients for the activated complexes across the energy barrier are equal. This seems to be a very logical conclusion since the potential energy surfaces for isotopic activated complexes should be very similar. The same argument holds for the length of the top of the energy barrier, which is assumed to be the same for both isotopic molecules. These assumptions might not be quite correct, but would scarcely account for the present discrepancy between theory and experiment. The ratio of the velocities with which the activated complexes pass over the energy barrier is taken as the ratio of the reduced masses of the two pairs of atoms involved in the bond cleavage, or bond formation. This approximation for the effective mass of the complex in the direction of decomposition may not be sufficiently accurate. It also may not be correct to apply the Teller-Redlich product rule to isotopic molecules when these are in an activated state.

It would seem that more, accurate C^{13} and C^{14} isotope effect measurements are needed. This implies that both effects should be measured using a mass spectrometer. Reactions of compounds whose partition functions could fairly readily be determined would be the most desirable types for study. Simple, unimolecular bond cleavage reactions would seem to be the best reactions for further testing of the present theory, or any modified theory.

SUMMARY

1. The relative rates of alkaline hydrolysis of ethyl benzoate-carboxyl-C^{12} and ethyl benzoate-carboxyl-C^{14} were measured, and it was found that the carboxyl-C^{14} ester hydrolyzed about 14 percent more slowly than the carboxyl-C^{12} ester.

2. A significant C^{14} isotope effect was found to occur in acid hydrolysis of ethyl benzoate-carboxyl-C^{14} and in alkaline hydrolysis of tertiary butyl benzoate-carboxyl-C^{14}, but not in acid hydrolysis of tertiary butyl benzoate-carboxyl-C^{14}. These observations indicate that the mechanism for acid hydrolysis of tertiary butyl benzoate involves alkyl oxygen bond rupture rather than alkoxyl carbon bond rupture, and thus supports the mechanism suggested by Cohen and Schneider for this reaction.

3. It was found that no significant C^{13} isotope effect occurred in the decarboxylation of anthranilic acid, from which it is concluded that carbon carbon bond rupture is not the rate determining step in this reaction. The decarboxylation was found to be acid-catalyzed. The mechanism is therefore bimolecular and the attack of the solvated proton must be the rate determining step.

4. The C^{13} and C^{14} isotope effects in the decarboxylation of mesitoic acid were measured using a mass spectrometer. The C^{13} isotope effect found is in agreement with the value obtained previously by Bothner-By and Bigeleisen. The C^{14} isotope effect is approximately two and one half times the C^{13} effect, whereas theory predicts it should be twice the C^{13} isotope effect.

CLAIMS TO ORIGINAL RESEARCH

1. The C^{14} isotope effects in the four reactions, acid and alkaline hydrolysis of ethyl benzoate-carboxyl-C^{14}, and acid and alkaline hydrolysis of tertiary butyl benzoate-carboxyl-C^{14} were measured for the first time.

2. The results of the C^{14} isotope effect studies in the ester hydrolyses provide additional evidence for the validity of the mechanism of hydrolysis for tertiary butyl benzoate suggested by Cohen and Schneider.

3. The C^{13} isotope effect in the decarboxylation of anthranilic acid was measured for the first time.

4. The decarboxylation of anthranilic acid was found to be acid-catalyzed.

5. A detailed mechanism for the decarboxylation of anthranilic acid has been suggested.

6. The C^{13} and C^{14} isotope effects in the decarboxylation of mesitoic acid were measured together, using a mass spectrometer. This is the first time that C^{13} and C^{14} isotope effects have been determined in the same reaction using the same measuring technique for both.

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A STUDY OF C^{13} AND C^{14} ISOTOPE EFFECTS IN SOME UNIDIRECTIONAL PROCESSES

Abstract

The C^{14} isotope effect in acid and alkaline hydrolysis of both ethyl and tertiary butyl benzoate-carboxyl-C^{14} have been measured. Significant isotope effects were found in all the hydrolyses except acid hydrolysis of the tertiary butyl ester. These results are additional evidence for the suggestion of Cohen and Schneider that alkyl oxygen rather than normal acyl oxygen bond cleavage occurs when tertiary butyl benzoate is hydrolyzed in acid solution. No appreciable C^{13} isotope effect was found to occur in thermal, aqueous, or acid-catalyzed decarboxylation of anthranilic acid. From this observation and other experimental evidence, it is concluded that anthranilic acid decarboxylates by a bimolecular process in which the attack of the hydrogen ion is rate controlling. It is further suggested that the hydrogen ion attack is on the switterion at the c carbon position. Both the C^{13} and the C^{14} isotope effect in the decarboxylation of mesitoic acid were measured using a mass spectrometer. The C^{14} isotope effect was found to be two and one half times the magnitude of the C^{13} isotope effect instead of twice as present theory predicts.