NITROGEN-15 ISOTOPE EFFECTS IN BOND FORMATION REACTIONS

NITROGEN-15 ISOTOPE EFFECTS
IN
BOND FORMATION REACTIONS

BY

ERNEST REGINALD HAYES, B.Sc., M.Sc.

A Thesis
Submitted to the Faculty of Arts and Science
in Partial Fulfillment of the Requirements
for the Degree
Doctor of Philosophy

McMaster University
October 1958

DOCTOR OF PHILOSOPHY (1958)
(Chemistry)

McMASTER UNIVERSITY
Hamilton, Ontario.

TITLE: NITROGEN-15 ISOTOPE EFFECTS IN BOND-FORMATION REACTIONS

AUTHOR: Ernest Reginald Hayes, B.Sc. (Acadia University)
M.Sc. (Dalhousie University)

SUPERVISOR: Dr. A.N. Bourns

NUMBER OF PAGES: ix, 209.

SCOPE AND CONTENTS:

Nitrogen-15 isotope effects have been measured in the simple bond formation reactions of triethylamine with methyl iodide, triethylamine with ethyl iodide and trimethylamine with ethyl bromide. Tests have been performed to establish that the reactions were unidirectional under the experimental conditions and that the chemical procedures used to convert the quaternary ammonium salts to molecular nitrogen proceeded quantitatively. The mass spectrometer data were examined by rigorous statistical tests. At 6°C. the values of the ratio of rate constants, k_{14}/k_{15}, were: triethylamine-methyl iodide, 1.0009 ±0.0005; triethylamine-ethyl iodide, 1.0007 ±0.0006; trimethylamine-ethyl bromide, 0.9994 ±0.0006. For the reaction of triethylamine with ethyl iodide the value of k_{14}/k_{15} increased slightly with increasing temperature. The results have been discussed in terms of the Bigeleisen theory of isotope effects with particular reference to the correct form for the effective mass term.

ACKNOWLEDGMENTS

The author is indebted to Dr. A.N. Bourns who supervised this research program. Dr. J.D. Bankier provided invaluable assistance in the statistical analyses of the mass spectrometer results. The writer wishes to thank Dr. H.G. Thode for helpful discussions and Dr. W.H. Fleming for assistance in the mass spectrometer analyses. The financial assistance received from the Research Council of Ontario and Canadian Industries Limited is gratefully acknowledged.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

TABLE OF CONTENTS (Continued)

DISCUSSION

APPENDIX I

APPENDIX II

LIST OF TABLES

LIST OF TABLES (Continued)

LIST OF FIGURES

GENERAL INTRODUCTION

Hydrogen isotope effects in unidirectional reactions have been recognized for many years. Until recently, however, it was believed that only insignificant isotope effects occurred during unidirectional reactions with the isotopic atoms of the higher elements. The first significant isotopic fractionation of an element other than hydrogen was observed by Stevenson _et al_ in 1948 during an investigation of the thermal cracking of propane-1-C^{13}(1). Since that time many other instances of isotope effects among the light elements in unidirectional reactions have been reported.

Both intramolecular and intermolecular isotope effects have been observed. An intramolecular isotope effect is defined by the relative rates with which two isotopic atoms, contained in similar functional groups of an otherwise symmetrical molecule, undergo the same reaction. The intermolecular isotope effect represents the relative rates of reaction of two isotopic atoms located in a similar functional group of different molecules of the same compound.

Isotope effects in unidirectional reactions, involving isotopes other than those of hydrogen, were first reported by Stevenson _et al_(1). While investigating the thermal cracking of propane-1-C^{13} these workers found that the C^{12}-C^{12} bonds of an unlabeled molecule were ruptured 8 per cent more frequently than the C^{12}-C^{13} bonds of a labeled molecule. These authors also observed a similar effect of 20 per cent during the dissociation of propane-1-C^{13} in the mass spectrometer(2). In a study of the decarboxylation of malonic acid, labeled with C^{14} in one carboxyl group, Yankwich and Calvin(3) found an intramolecular isotope effect of 12 per cent. Smaller C^{13} isotope effects were observed by Bigeleisen and Friedman(4) in the decarboxylation of malonic acid of normal isotopic content. They found intramolecular and intermolecular isotope effects of 2.0 and 3.7 per cent, respectively. Lindsay, McElcheran and Thode(5) investigated the C^{13} isotope effects in the decomposition of normal oxalic acid in sulfuric acid. Intramolecular and intermolecular C^{13} isotope effects of 3.2 and 3.4 per cent, respectively, were reported for this reaction. Since these first studies, isotope effects involving carbon, nitrogen, oxygen and sulfur isotopes have been studied in many other systems.

A theoretical description of the effect of isotopic substitution on the rate of unidirectional chemical reactions has been developed by Bigeleisen from the postulates of absolute rate theory and statistical mechanics(6). The Bigeleisen equation, from which the ratio of rate constants of isotopic molecules can be calculated, is as follows:

$$\frac{k_1}{k_2} = s\left(\frac{m_2^*}{m_1^*}\right)^{\frac{1}{2}} \left[1 + \sum_{1}^{3n-6} G(u_i)\Delta u_i - \sum_{1}^{3n'-6} G(u_i^{\neq})\Delta u_i^{\neq}\right], \qquad (1)$$

where s is a symmetry number, m^* is the effective mass of the molecule along the reaction coordinate, the subscripts 1 and 2 refer to the light and heavy isotopic molecules, respectively, $G(u_i)$ is a function defined by Bigeleisen and Mayer(7), Δu_i equals $(hc/kT)(\omega_{1i} - \omega_{2i})$, where the ω's are vibrational frequencies, and $\neq$ refers to the activated complex. s is unity except in reactions in which there are two or more identical atoms of the isotope in question in the same molecule. The term, $(m_2^*/m_1^*)^{\frac{1}{2}}$, expresses the ratio of the number of isotopic molecules in the transition state undergoing reaction per unit time and for simple bond rupture or bond formation processes may be calculated as the reduced mass across the bond in question. The quantity in the square brackets, which is related to the normal vibrations of the molecule and the activated complex, quantitatively describes the free energy effects.

The treatment of intermolecular isotope effects is complex because all terms of the Bigeleisen equation must be considered. For bond rupture processes the free energy term for the initial state is greater than that for the transition state. Since the effective mass and the square bracket terms act in the same direction, the theory predicts that the isotope effect will have some value greater than the effective mass term. An upper limit for the isotope effect can be computed by assuming that the bond is completely broken in the transition state and that the frequencies of the other bonds in the molecule are not affected by the bond rupture. The magnitude of many isotope effects are in reasonable agreement with the values calculated using these assumptions. Further, the prediction that the isotope effect will decrease in magnitude with increasing reaction temperature has been verified experimentally.

For simple bond formation reactions, the free energy term for the transition state is greater than that for the initial state. Since the square bracket term tends to cancel the effective mass term, the Bigeleisen theory predicts that the isotope effect will have some value less than that of the effective mass term and that the lighter isotope will be favored by an increase in reaction temperature, that is, the value of k_1/k_2 will tend to that of the effective mass term as an upper limit as the reaction temperature is raised. As no study of isotope effects in strictly unidirectional bond formation processes has yet been reported, neither of the above predictions has received experimental verification.

Very few isotope effects in bond formation reactions have been studied and these, with one exception, have been stepwise processes in which the bond formation step was not necessarily rate determining. The bisulphite addition reaction of aldehydes and ketones, for which Sheppard, Bader and Bourns found a very small sulphur isotope effect, is the only one step bond formation reaction which has been investigated(8). Unfortunately, under their experimental conditions this reaction was not strictly unidirectional although the authors attempted to minimize the reverse reaction by removing the precipitated product from the reaction system as rapidly as possible. The experimental values of the isotope effect were in good agreement with the Bigeleisen model.

The main objective of the present investigation was to test the predictions of the Bigeleisen theory with respect to the magnitude and temperature dependence of isotope effects in reaction systems which are strictly unidirectional bond formation processes. As a secondary objective one bond formation reaction was studied to secure additional information as to the correct form of the effective mass term in the Bigeleisen equation. The reactions of alkyl amines with alkyl halides,

$$R_3N + R'X \longrightarrow (R'R_3N)^{+}X^{-}$$

were selected because with respect to the nitrogen atom they are simple bond formation processes and under ordinary conditions they proceed irreversibly to product. The nitrogen isotope effect, k_{14}/k_{15}, was determined using amines of normal isotopic abundances.

The reactions studied were:

(1) triethylamine and methyl iodide,

(2) triethylamine and ethyl iodide,

(3) triethylamine and isopropyl iodide,

(4) trimethylamine and ethyl bromide.

Since the reaction of triethylamine with methyl iodide is very fast, the isotope effect was determined at only one temperature, 8°C. The reaction of triethylamine with ethyl iodide was studied over the temperature range 6 to 78°C. An attempt was made to measure the isotope effect in the reaction of triethylamine with isopropyl iodide at 61°C. The isotope effects in the reaction, trimethylamine and ethyl bromide, were measured at 6 and 25°C.

As previously stated the reaction of trimethylamine with ethyl bromide was studied in an attempt to obtain further information as to the correct form for the effective mass term in bond formation processes. If the effective mass term is considered simply as the reduced mass of the bond being formed, as is the case for simple bond rupture processes, then this term would be the same for all reactions involving the same isotopic atoms. However, if the masses of the reacting molecules themselves should be considered in evaluating the effective mass term, this factor will be different for each reaction system. The larger the effective mass term, the larger the isotope effect expected in a bond formation process, assuming the free energy contributions of the activated complexes to be approximately the same. The trimethylamine - ethyl bromide reaction was selected for study because an effective mass term calculated as the reduced mass of the reacting molecules would be significantly larger than the corresponding mass terms for the other systems studied. If the isotope effect is appreciably larger in the trimethylamine - ethyl bromide system than in the other reactions, it would suggest that the masses of the reacting molecules should be used in the calculation of the effective mass term. Conversely, an isotope effect of approximately the same magnitude in all the bond formation reactions might be taken to indicate that the effective mass term may be simply treated as the reduced mass of the bond being formed.

HISTORICAL INTRODUCTION

The theoretical treatment of the relative reaction velocities of isotopic molecules has been developed by Bigeleisen(6) from the basic postulates of absolute rate theory which treats the problem of reaction rates from the viewpoint of statistical mechanics. The statistical mechanics and absolute rate theory necessary for the development of the Bigeleisen equation will be reviewed briefly before treating the Bigeleisen model.

Statistical Mechanics (9)

The basic problem of statistical mechanics is the determination of the probability of the occurrence of any particular state of a system. The fundamental equations describing the statistical behaviour of an ensemble consisting of a large number of particles were first derived by Boltzmann. The Boltzmann distribution law was derived for a system of total energy E, containing n identical and distinguishable particles, on the assumption that the allowed energy levels, $\epsilon_1, \epsilon_2, \ldots, \epsilon_i$, were known from quantum mechanics. The Boltzmann distribution law, which defines the distribution of the total energy among n particles in the allowed energy levels, may be expressed as

$$n_i = \text{constant} \times g_i e^{-\frac{\epsilon_i}{kT}}, \tag{2}$$

where n_i is the number of particles assigned to the ϵ_i energy level, g_i is the degeneracy or statistical weight of the state, ϵ_i is the i^{th} energy level, k is the Boltzmann constant, and T is the absolute temperature.

While the above distribution law is only an approximation of the correct quantum mechanical distribution law, it can frequently be used without serious error. The inexactness in the Boltzmann law is principally due to the assumptions that both the position and velocity of a particle can be defined exactly and that identical particles are distinguishable.

Since the probability that any given molecule shall have energy ϵ in any quantum state that is g - fold degenerate is proportional to the quantity $g_i e^{-\frac{\epsilon_i}{kT}}$, the total probability of the occurrence of a particular molecular species is proportional to the sum of the terms. This sum, defined by

$$q = \sum_i g_i \, e^{-\frac{\epsilon_i}{kT}} , \qquad (3)$$

is called the partition function for the molecule. In principal, the values of the partition functions can be derived from spectroscopic data and these values can then be used to calculate all thermodynamic quantities and reaction rate constants.

The complete partition function, Q, for any species may include terms for electronic, vibrational, rotational and translational energy. In evaluating the complete partition function the simplifying assumption is made that the different forms of energy of the system are essentially independent and that the total energy corresponding to a given energy level may be written as

$$\epsilon_i = \epsilon_{i\,\text{electronic}} + \epsilon_{i\,\text{vibrational}} + \epsilon_{i\,\text{rotational}} + \epsilon_{i\,\text{translational}}$$

where the terms on the right represent the different types of energy corresponding to the ith state. The complete partition function, therefore, becomes

$$q = q_{\text{electronic}}\ q_{\text{vibrational}}\ q_{\text{rotational}}\ q_{\text{translational}}$$

This definition of the complete partition function is only an approximation since a change in vibrational energy leads to an alteration in the spacing of the rotational levels and, similarly, the vibrational and rotational levels are both affected by the electronic state of the molecule. However, this approximation is satisfactory for many purposes.

The electronic contributions to the complete partition function will be neglected in the following discussion. For most molecules only the lowest electronic level is occupied except at very high temperatures. Consequently, the electronic ground states are singlets for such molecules, and the value of the electronic partition function is unity. The contribution of the nuclear energy to the complete partition function is generally included with the rotational partition function, since the statistical weight of each rotational level is determined by the spin of the nuclei in the molecule as well as by the rotational quantum numbers. Such effects, however, lie outside the scope of this discussion.

The translational energy of a molecule can be treated as an entity composed of three independent components at right angles to each other. The quantum mechanical expression for the translational energy per molecule for one degree of freedom in a box of side a is

$$\epsilon_{tr} = \frac{n^2h^2}{8ma^2} \quad , \qquad (4)$$

where n is a quantum number, h is Planck's constant, m is the mass, and a is equivalent to the quantum mechanical energy in any one of the three directions. Therefore the translational function for one degree of freedom is:

$$q_{tr} = \sum_{n=0}^{\infty} g_{i\ tr} \; e^{\frac{-n^2h^2}{8ma^2kT}} \quad . \qquad (5)$$

Since the translational energy states are nondegenerate the statistical weight, $g_{i\ tr}$, is unity. The energy levels are so closely spaced that they can be considered continuous, hence, the summation can be replaced by an integration, which gives

$$q_{tr} = \frac{(2\pi mkT)^{\frac{1}{2}}a}{h} \quad . \qquad (6)$$

Similar equations can be derived for the other two degrees of freedom. The partition function for the three degrees of translational energy is the product of the three terms,

$$q_{tr} = \frac{(2\pi mkT)^{\frac{3}{2}}v}{h^3} \quad , \qquad (7)$$

where v is the volume of the box. When using the partition function to calculate thermodynamic quantities for one mole of ideal gas, v becomes the molar volume.

The vibrational partition function is usually derived for a diatomic molecule and it is assumed that the vibrational partition function of a polyatomic molecule can be expressed by a formula of the same type. The equation for the vibrational energy is derived on the assumption that a diatomic molecule can be represented by a linear harmonic oscillator whose energy is given by

$$\epsilon_{v} = hc\omega(v + \tfrac{1}{2}), \tag{8}$$

where ω in cm^{-1} is the fundamental vibration frequency of the molecule in its ground state and v is the vibrational quantum number which may have any integral value including zero. The zero point energy, which is the vibrational energy for $v = o$, is

$$\epsilon_o = \tfrac{1}{2}\, hc\omega \quad . \tag{9}$$

Since the vibrational energy for a diatomic molecule is defined as $\epsilon_v - \epsilon_o$,

$$\epsilon_{vib} = hcv\omega. \tag{10}$$

The vibrational partition function, therefore, is given by

$$q_{vib} = \sum_{v=0}^{\infty} g_{i\,vib}\, e^{-\frac{hcv\omega_i}{kT}} . \tag{11}$$

For most molecules $g_{i\,vib}$ is unity. Expansion of equation (11) gives

$$q_{vib} = (1 - e^{-\frac{hc\omega_i}{kT}})^{-1} \quad . \qquad (12)$$

As the temperature is raised a diatomic molecule tends to approach the behaviour described by a linear harmonic oscillator, and at appreciable temperatures the agreement between calculated and observed values is good.

For a polyatomic molecule with 3n-x vibrational degrees of freedom the vibrational partition function may be written

$$Q_{vib} = \prod_{i=1}^{3n-x} (1 - e^{-\frac{hc\omega_i}{kT}})^{-1} \qquad (13)$$

the product being taken over all degrees of freedom. There is a term in the above product for each of the vibrational modes of the molecule. The number of terms included in the product depends on the number and nature of the normal vibrations, e.g., for a nonlinear polyatomic molecule 3n-6 vibrational modes are required to describe in Cartesian coordinates the vibrations of the n nuclei in the molecule.

If the reference zero is taken as the bottom of the potential energy curve rather than the zero point energy of the molecule, the equations for the vibrational partition functions become

$$q_{vib} = \frac{e^{-\frac{hc\omega_i}{2kT}}}{1 - e^{-\frac{hc\omega_i}{kT}}} \qquad (12')$$

and

$$Q_{vib} = \prod_{i=1}^{3n-x} \frac{e^{-\frac{hc\omega_i}{2kT}}}{1 - e^{-\frac{hc\omega_i}{kT}}} \qquad (13')$$

Since the exact determination of the rotational partition function is complicated for even simple molecules, a number of simplifying assumptions are made. If the vibrational and rotational energies are assumed to be independent, a diatomic molecule can be treated as a rigid rotator with a free axis. The rotational energy for a single molecule is then given by

$$\epsilon_{rot} = J(J+1)\,\frac{h^2}{8\pi^2 I}\,, \tag{14}$$

where I is the moment of inertia, h is Planck's constant, and J is the rotational quantum number which can have zero or integral values. For every value of J there are $2J + 1$ eigenstates corresponding to approximately the same rotational energy levels. Thus each rotational level has a degeneracy of $2J + 1$ and the expression for the rotational partition function is

$$q_{rot} = \sum_{J=0}^{\infty} (2J+1)\, e^{-J(J+1)\rho}\,, \tag{15}$$

where

$$\rho = \frac{h^2}{8\pi^2 I k T}\,. \tag{16}$$

If ρ is less than unity, which is the case at all except the lowest temperatures for all elements except hydrogen, this summation can be expanded to the series

$$q_{rot} = \frac{1}{\rho}\left(1 + \frac{\rho}{3} + \frac{\rho^2}{15} + \frac{4\rho^3}{315} + \cdots\right). \tag{17}$$

If ρ is less than 0.05, which is true for nearly all substances at moderately high temperatures, all terms beyond the first can be neglected and the rotational partition function for a rigid diatomic molecule is given by

$$q_{rot} = \frac{1}{\rho} = \frac{8\pi^2 IkT}{h^2} \quad . \tag{18}$$

This expression for the rotational partition function is incomplete in that the effect of nuclear spin and the symmetry number are missing. When these factors are included the rotational partition function becomes

$$q_{rot} = g_{i\,nuc} \frac{8\pi^2 IkT}{s h^2} \quad , \tag{19}$$

where $g_{i\,nuc}$ is the statistical weight or degeneracy due to the different orientations of the nuclei in the diatomic molecule and s, the symmetry number, represents the number of indistinguishable orientations of a particular molecule as a result of rotation. For polyatomic molecules analagous expressions have been developed for the rotational partition function, the exact form depending on the configuration of the molecule.

Absolute Rate Theory (10)

Absolute rate theory is based on the postulate that the reaction coordinate, which describes any atomic or molecular process, passes through a potential energy maximum. The molecular configuration of the reacting species at the top of this energy barrier is called the activated complex. These complexes are arbitrarily considered to exist in a potential box of length δ which lies along the reaction coordinate. An activated complex has all of the properties of a normal molecule except that the normal vibration frequency in the coordinate of decomposition has an imaginary value, that is, it is unstable to displacement in the vibrational mode associated with the bond being broken; in this coordinate the activated complex decomposes. If the energy barrier in the vicinity of the activated complex is relatively flat, the degree of freedom in the decomposition coordinate may be considered statistically as a one-dimensional translation.

To simplify the calculation of reaction rates, it is assumed that the initial reactants are always in equilibrium with the activated complex and that this equilibrium can be treated by statistical mechanics. It is further assumed that the activated complexes decompose at a definite rate and that the concentration of activated complexes is not appreciably disturbed by the reaction.

The net rate with which reaction occurs is determined by the average velocity with which the activated complexes cross the top of the energy barrier. It can be shown that, $\bar{x}$, the average rate of passage of activated complexes over the barrier in one direction along the coordinate of decomposition, is given by the expression

$$\bar{x} = \left(\frac{kT}{2\pi m^*}\right)^{\frac{1}{2}} , \qquad (20)$$

where m^* is the effective mass of the complex in the same direction. The average time, τ , required for the activated complex to cross the energy barrier, is equal to the length of the top of the barrier, δ , divided by the average rate of crossing, $\bar{\dot{x}}$. Therefore,

$$\tau = \frac{\delta}{\bar{\dot{x}}} = \delta \left(\frac{2\pi m^*}{kT} \right)^{\frac{1}{2}} \quad . \tag{21}$$

If $c^{\ddagger}$ is the number of activated complexes per unit volume lying in the length δ of the coordinate of decomposition, the quantity $c^{\ddagger}/\tau$ gives the number of complexes crossing the barrier per unit volume in unit time. If every complex that moves across the barrier decomposes to products, the rate of the reaction is given by

$$\text{Rate of reaction} = \frac{c^{\ddagger}}{\tau} = c^{\ddagger} \left(\frac{kT}{2\pi m^*} \right)^{\frac{1}{2}} \frac{1}{\delta} \; . \tag{22}$$

The rate also may be expressed in the more conventional form

$$\text{Rate of reaction} = k \, C_A C_B \cdots , \tag{23}$$

where k is the specific reaction rate constant, and C_A and C_B etc., are the concentrations of the reacting species. Combination of equations (22) and (23) gives the following expression for the rate constant ,

$$k = \frac{c^{\ddagger}}{C_A C_B \cdots} \left(\frac{kT}{2\pi m^*} \right)^{\frac{1}{2}} \frac{1}{\delta} \quad . \tag{24}$$

The transmission coefficient κ must be introduced into equation (24) to allow for the possibility that not all the activated complexes reaching the top of the energy barrier and moving along the reaction coordinate proceed to products.

κ represents the possibility that systems with sufficient energy to reach the activated state may be reflected back to the initial state because of the curvature of the potential energy surface. Introduction of this factor into equation (24) gives

$$k = \kappa \frac{C^{\ddagger}}{C_A\ C_B \cdots} \left(\frac{kT}{2\pi m^*} \right)^{\frac{1}{2}} \frac{1}{\delta} \ . \tag{25}$$

The transmission coefficient is ordinarily considered to be unity because of the difficulty of calculating it from the potential energy surface. This is believed to be a good approximation for most reaction systems. An additional correction is necessary to allow for the possibility of tunneling through the energy barrier. With the exception of the hydrogen isotopes, however, this leakage is negligible for most systems and ordinarily no correction is applied.

Derivation of the Bigeleisen Equation

The Bigeleisen equation for the calculation of the relative rate constants for competitive reactions of isotopic molecules is based on the postulates of absolute rate theory and statistical mechanics(6). This derivation reduces to a minimum the number of properties of the activated complex which must be evaluated before isotope effects can be calculated for unidirectional processes. In this derivation use is made of a function derived by Bigeleisen and Mayer(7), who showed that the ratio of the complete partition function for two isotopic molectules can be expressed as a simple function of the vibrational energy levels of the two molecules. The use of this function greatly simplifies the calculations of both isotope effects and equilibrium constants and, at the same time, clearly shows that the chemical separation of isotopes is a quantum effect.

The problem of isotope effects cannot be treated by classical mechanics since classical theory predicts that isotopic fractionation is not possible in a chemical reaction. This can best be illustrated by considering the classical derivation of equilibrium constants. The classical expression for the partition function is

$$h^f Q = \frac{1}{s} \iiint \cdots \iint e^{-\frac{H(p,q)}{kT}} \quad dq_1, \ldots, dp_f \qquad (26)$$

where h^f is Planck's constant for a system having f degrees of freedom, s is a symmetry number, Q is the complete partition function, and H is the Hamiltonian of the momenta p and coordinates q necessary to define the f degrees of freedom of the constituent atoms. Since the momenta of the atoms occur only as kinetic energy, they may be expressed by quadratic functions of the form $p^2/2\,m$. Then integration over the momenta leads to

$$Q = \frac{1}{s} \prod_i \frac{(2\pi m_i kT)^{\frac{3}{2}}}{h^2} \iiint \cdots \int e^{-\frac{u}{KT}} \, dq_1, \ldots, dq_k \qquad (27)$$

The potential energies of molecules which differ only in isotopic composition are alike. Therefore, the ratio of the partition functions of two isotopic molecules reduces to the inverse ratio of the symmetry numbers multiplied by the mass ratio of the different isotopes raised to the three-halves power, i.e.,

$$\frac{Q}{Q'} = \frac{s'}{s}\left(\frac{m}{m'}\right)^{\frac{3}{2}} . \qquad (28)$$

Neither of these terms can lead to an isotopic enrichment. The ratio of the symmetry numbers merely represents the relative probabilities of forming symmetrical and unsymmetrical molecules. The ratio of the masses of the isotopes cancel in any chemical equilibrium. Classical theory, therefore, predicts that the value of the equilibrium constant in any exchange reaction is unity.

Quantum theory, on the other hand, successfully predicts isotopic fractionations and also permits calculation of the magnitude of these fractionations. Instead of using the unsatisfactory classical mass ratio $(m/m')^{3/2}$ in the calculation of equilibrium constants in isotope exchange reactions, Bigeleisen and Mayer defined a new function,

$$f = \frac{Q}{Q'} \prod_i \left(\frac{m_{i(1)}}{m_{i(2)}}\right)^{\frac{3}{2}} . \qquad (29)$$

The omission of the classical mass ratio is equivalent to calculating the ratio of the equilibrium constants for the dissociation of the two isotopic atoms into molecules. The product $(s/s')f$ in classical mechanics is unity which implies no isotopic separation. In quantum mechanics, however, this product has a value other than one.

As a first approximation, the complete partition function of quantum mechanics may be written as the product of the translational, rotational and vibrational partition functions. The translational partition function is the same in both quantum and classical mechanics at all temperatures. The classical form of the rotational partition function may be used for all elements, except hydrogen, at room temperature. Therefore, the factor which leads to isotopic separation is to be found in the vibrational partition function. The classical vibration partition function is equivalent to the value given by quantum mechanics only at high temperatures.

Neglecting anharmonicity the vibrational partition function is given by

$$Q_{vib} = \prod_i \frac{e^{-\frac{hc\omega_i}{2kT}}}{1 - e^{-\frac{hc\omega_i}{kT}}} \qquad (13')$$

or

$$Q_{vib} = \prod_i \frac{e^{-\frac{u_i}{2}}}{1 - e^{-u_i}} \qquad (30)$$

where

$$u_i = \frac{hc\omega_i}{kT}. \qquad (31)$$

Since the value of f depends on the molecular vibrations only, equation (29) becomes

$$\frac{s}{s'} f = \prod_i \frac{u_i}{u_i'} \; \frac{e^{-\frac{u_i}{2}}}{1 - e^{-u_i}} \; \frac{1 - e^{-u_i'}}{e^{-u_i'/2}} \; . \qquad (32)$$

The term u_i' may be replaced by $u_i + \Delta u_i$ where the primed molecule contains the lighter isotope. Then

$$\Delta u_i = \frac{hc\,(\omega_{1i} - \omega_{2i})}{kT} \tag{33}$$

and

$$\frac{s}{s'} f = \frac{u_i}{u_i + \Delta u_i}\; e^{-\frac{\Delta u_i}{2}}\, \frac{(1 - e^{-(u_i + \Delta u_i)})}{(1 - e^{-u_i})} \; . \tag{34}$$

If Δu_i is small, which is true for all isotopes except those of hydrogen, equation (34) leads to

$$\frac{s}{s'} f = 1 + \sum_i \left(\frac{1}{2} - \frac{1}{u_i} + \frac{1}{e^{u_i} - 1} \right) \Delta u_i \; . \tag{35}$$

To facilitate calculations a function G has been defined as

$$G = \frac{1}{2} - \frac{1}{u_i} + \frac{1}{e^{u_i} - 1} \tag{36}$$

and values of G have been calculated and tabulated from $u = 0$ to $u = 25$(7). This expression for f is used for most calculations, i.e.,

$$f = \frac{s'}{s} \left(1 + \sum_i G(u_i)\, \Delta u_i\right) \; . \tag{37}$$

In the Bigeleisen model of isotope effects in unidirectional reactions, rate constants for the isotopic species are derived from absolute rate theory. The model is then greatly simplified by introducing the f functions, defined originally for isotopic exchange reactions.

Consider the reactions,

$$A_1,\ B_1,\ C \ldots \longrightarrow P_1 \tag{ii}$$

$$A_2,\ B_1,\ C \ldots \longrightarrow P_2 \; , \tag{iii}$$

where A_1 and A_2 are isotopic molecules. As was discussed earlier, the corresponding reaction rate constants are given by

$$k_1 = \kappa_1 \frac{C_1^{\ddagger}}{C_{A_1} C_{B_1} \cdots} \left(\frac{kT}{2\pi m_1^*} \right)^{\frac{1}{2}} \frac{1}{\delta_1} \tag{39}$$

and

$$k_2 = \kappa_2 \frac{C_2^{\ddagger}}{C_{A_2} C_{B_2} \cdots} \left(\frac{kT}{2\pi m_2^*} \right)^{\frac{1}{2}} \frac{1}{\delta_2} , \tag{40}$$

where κ is the transmission coefficient, C is the concentration of the initial reactants, $C^{\ddagger}$ is the concentration of the activated complex, m^* is the effective mass of the activated complex along the coordinate of decomposition, and δ is the length of the top of the potential energy barrier which the complex traverses. Since the potential energy surfaces for isotopic molecules are the same to a high degree of approximation

$$\delta_1 = \delta_2$$

Therefore, the ratio of rate constants for isotopic molecules is given by

$$\frac{k_1}{k_2} = \frac{\kappa_1}{\kappa_2} \frac{C_1^{\ddagger}}{C_2^{\ddagger}} \frac{C_{A_2}}{C_{A_1}} \left(\frac{m_2^*}{m_1^*} \right)^{\frac{1}{2}} . \tag{41}$$

Replacing the ratio of the concentrations of the individual molecules by the corresponding complete partition functions gives

$$\frac{k_1}{k_2} = \frac{\kappa_1}{\kappa_2} \frac{Q_1^{\ddagger}}{Q_2^{\ddagger}} \frac{Q_{A_2}}{Q_{A_1}} \left(\frac{m_2^*}{m_1^*} \right)^{\frac{1}{2}} . \tag{42}$$

As in equilibrium exchange reactions, the ratio of the complete partition functions of the two isotopic molecules reduces to equation (29)

$$f = \frac{Q}{Q'} \prod_i \left(\frac{m_{i(1)}}{m_{i(2)}} \right)^{\frac{3}{2}} ,$$

which is simply a function of the vibrational energy levels of the two molecules. Replacing the complete partition functions of equation (42) by the function f gives

$$\frac{k_1}{k_2} = \frac{\kappa_1}{\kappa_2} \frac{f}{f^{\ddagger}} \left(\frac{m_2^*}{m_1^*} \right)^{\frac{1}{2}} . \qquad (43)$$

The terms f and $f^{\ddagger}$ may now be replaced by equation (37), which is valid when Δu_i is small, hence,

$$\ln \frac{k_1}{k_2} = \ln \frac{\kappa_1}{\kappa_2} \frac{s_1}{s_2} \frac{s_2^{\ddagger}}{s_1^{\ddagger}} + \frac{1}{2} \ln \left(\frac{m_2^*}{m_1^*} \right) + \ln \left[\frac{1 + \sum_i^{3n-6} G(u_i) \Delta u_i}{1 + \sum_i^{3n'-6} G(u_i^{\ddagger}) \Delta u_i^{\ddagger}} \right]$$

(44)

which may also be expressed as

$$\frac{k_1}{k_2} = \frac{\kappa_1}{\kappa_2} \frac{s_1}{s_2} \frac{s_2^{\ddagger}}{s_1^{\ddagger}} \left(\frac{m_2^*}{m_1^*} \right)^{\frac{1}{2}} \left[1 + \sum_i^{3n-6} G(u_i) \Delta u_i - \sum_i^{3n'-6} G(u_i^{\ddagger}) \Delta u_i^{\ddagger} \right]$$

(45)

where the subscripts 1 and 2 refer to the light and heavy isotopes, respectively, the symbol $\ddagger$ refers to the activated state, m^* is the effective mass of the molecule along the reaction coordinate, s is a symmetry number, and κ is the transmission coefficient. The quantity in the square brackets quantitatively describes the free energy effects associated with the initial and activated states. The factor $(m_2^*/m_1^*)^{\frac{1}{2}}$ gives the ratio of the number of molecules in the transition state undergoing reaction per unit time.

A correction for the tunnel effect should be made to arrive at the exact form of the Bigeleisen equation. Since this correction is negligible for the systems included in this discussion, it will be omitted.

The exact values of the transmission coefficients are unknown, but it is believed that they can be set equal to each other without introducing appreciable error. The symmetry numbers are unity unless there are two or more identical atoms of the isotope in question in the reacting molecule. When these simplifications are made, the Bigeleisen equation becomes

$$\frac{k_1}{k_2} = \left(\frac{m_2^*}{m_1^*}\right)^{\frac{1}{2}} \left[1 + \sum_i^{3n-6} G(u_i)\,\Delta u_i - \sum_i^{3n'-6} G(u_i^{\ddagger})\,\Delta u_i^{\ddagger}\right] . \qquad (1)$$

In reactions involving simple bond rupture, Slater has shown that the effective mass term may be calculated as the reduced mass of an imaginary diatomic molecule composed of the atoms whose bond is broken in the rate determining step(11). The fundamental vibration frequency of the bond undergoing reaction is required to evaluate $G(u_i)\,\Delta u_i$. For a diatomic molecule the fundamental vibration frequency ν_1 is given by

$$\nu_1 = \frac{1}{2\pi}\sqrt{\frac{k_1}{\mu}} \quad , \qquad (46)$$

where k_1 is the force constant of the molecule and μ is the reduced mass. The shift in the frequency due to isotopic substitution is given by the inverse ratio of the reduced masses raised to the one-half power, i.e.,

$$\frac{\nu_1}{\nu_2} = \left(\frac{\mu_2}{\mu_1}\right)^{\frac{1}{2}} \quad . \qquad (47)$$

These equations cannot be used for the accurate determination of the vibration frequency of a complex molecule. Frequencies derived in this manner are only approximations since the normal mode of vibration can no longer be ascribed to the stretching of a single bond.

In the derivation of the Bigeleisen equation there are the usual assumptions inherent in the transition state hypothesis. Specifically, it is assumed that there is no interaction between the rotational and vibrational energy levels and that the normal vibrations of the molecule are harmonic in character. Interactions between other atoms in the molecule and the bond being ruptured are disregarded in the evaluation of the effective mass term. Moreover, the crossing of the energy barrier is treated as a translation while the partition function corresponding to this motion is treated as a vibration. In a later paper Wolfsberg and

Bigeleisen show that this treatment is satisfactory since the usual potential surface leads to an imaginary frequency which behaves like a translation(12, 13). The difference between the original Bigeleisen derivation and a more formal treatment, in which both motion across the barrier and its associated partition function are treated as a translation, is equal to the Wigner tunnel correction, which is negligible except in the case of the hydrogen isotopes.

The Bigeleisen equation, as applied to simple unidirectional reactions, consists of a temperature independent term $(m_2^*/m_1^*)^{\frac{1}{2}}$ and a free energy term dependent on temperature,

$$1 + \sum_i^{3n-6} G(u_i)\, \Delta u_i - \sum_i^{3n'-6} G(u_i^{\ddagger})\, \Delta u_i^{\ddagger} \quad .$$

Depending on the reaction system these terms may act in the same direction producing a large isotope effect or tend to cancel each other with a resultant small isotope effect. In general, however, the lighter of two isotopic molecules will have the greatest rate constant since the effective mass term is always greater than unity.

If the reacting molecules are more "tightly bound" in the initial state then in the activated complex, i.e.,

$$\sum_i^{3n-6} G(u_i)\, \Delta u_i \; > \; \sum_i^{3n'-6} G(u_i^{\ddagger})\, \Delta u_i^{\ddagger} \quad ,$$

the free energy term is greater than one and acts in the same direction as the effective mass term. The largest isotopic separation occurs when the bond is completely broken in the activated complex, that is, when

$$\sum_{i}^{3n'-6} G(u_i^{\ddagger})\,\Delta u_i^{\ddagger} = 0$$

and

$$\frac{k_1}{k_2} = \left(\frac{m_2^*}{m_1^*}\right)^{\frac{1}{2}} \left[1 + \sum_{i}^{3n-6} G(u_i)\,\Delta u_i \right] . \qquad (48)$$

These considerations apply to the case of bond rupture when the isotopic atoms occur in different molecules. For this type of reaction the theory predicts that the isotope effect will be temperature dependent, decreasing as the reaction temperature is raised.

A special case arises when a molecule, ABA , which is unsymmetrical only because of isotopic composition, can decompose by two alternate and equivalent paths. Since the ground state for both isotopes is the same molecule,

$$\sum_{i}^{3n-6} G(u_i)\,\Delta u_i = 0$$

If it is assumed that the isotopic atoms associated with the bond being ruptured are essentially free in the activated complex, then

$$\sum_{i}^{3n'-6} G(u_i^{\ddagger})\,\Delta u_i^{\ddagger} = 0$$

and the ratio of the rate constants is simply

$$\frac{k_1}{k_2} = \left(\frac{m_2^*}{m_1^*}\right)^{\frac{1}{2}} . \qquad (49)$$

In this case the theory predicts that the isotope effect is independent of temperature.

The final case arises when the molecules are more "tightly bound" in the activated complex than in the initial state, that is, when

$$\sum_{i}^{3n'-6} G(u_i^{\neq})\,\Delta u_i^{\neq} > \sum_{i}^{3n-6} G(u_i)\,\Delta u_i \ .$$

Then the value of the free energy term will be less than one and the isotope effect will have some value less than that of the effective mass term. The isotope effect will have its smallest value when the isotopic atoms are free in the initial state, i.e.,

$$\sum_{i}^{3n-6} G(u_i)\,\Delta u_i = 0$$

and

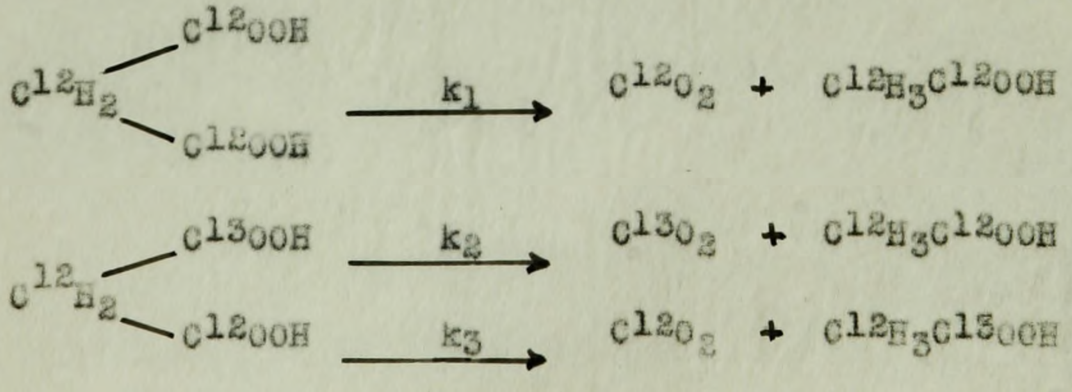
$$\frac{k_1}{k_2} = \left(\frac{m_2^*}{m_1^*}\right)^{\frac{1}{2}} \left[1 - \sum_{i}^{3n'-6} G(u_i^{\neq})\,\Delta u_i^{\neq}\right] \qquad (50)$$

This type of process is represented by bond formation reactions in which the isotopic atoms are present in different molecules. The theory is unable to predict which isotope will be favored in a bond formation reaction since this depends on the magnitude of the free energy term. Theoretically, it is possible that the free energy term could be sufficiently large that the heavy isotope would have a greater rate constant than the light isotope, i.e., $k_1/k_2 < 1$. The theory predicts that the isotope effects occurring in such systems will increase as the reaction temperature is increased. Since the value of the free energy term decreases with increasing temperature, at higher temperatures there is less cancellation of the effective mass term and the value of k_1/k_2 tends to that of the effective mass term as an upper limit.

The theoretical predictions of the Bigeleisen equation will be compared with the experimental results in the following discussion.

Correlation Between Experimental Results and Theory

Isotope effects in bond breaking reactions have been the object of considerable experimental study and both intramolecular and intermolecular isotope effects have been measured. Since the conventions commonly used to describe these effects were defined for the decarboxylation of malonic acid and since this is the system which has been studied the most intensively, the kinetic scheme for the decomposition of the normal acid is outlined below.



$$C^{12}H_2(C^{12}OOH)_2 \xrightarrow{k_1} C^{12}O_2 + C^{12}H_3C^{12}OOH \qquad \text{(iv)}$$

$$C^{12}H_2(C^{13}OOH)(C^{12}OOH) \xrightarrow{k_2} C^{13}O_2 + C^{12}H_3C^{12}OOH \qquad \text{(v)}$$

$$C^{12}H_2(C^{13}OOH)(C^{12}OOH) \xrightarrow{k_3} C^{12}O_2 + C^{12}H_3C^{13}OOH \qquad \text{(vi)}$$

The intramolecular isotope effect is the relative rate of rupture of C^{12}-C^{12} and C^{12}-C^{13} bonds in the decomposition of the dibasic acid singly-labeled in one carboxyl group. This effect is given by the ratio of rate constants k_3/k_2. Experimentally the value of k_3/k_2 may be obtained by comparing the isotopic abundance of the carbon dioxide from the complete thermal decomposition of malonic acid with that of the carboxyl groups in the starting material. The intermolecular isotope effect measures the relative rates of rupture of C^{12}-C^{12} and C^{12}-C^{13} bonds in unlabeled malonic acid and acid containing a C^{13} atom in one carboxyl group, respectively. The intermolecular effect is given by the ratio of rate constants $k_1/2\,k_2$. The experimental value of $k_1/2\,k_2$ is obtained by intercomparing the isotopic ratio of the carbon dioxide produced from a low percent decomposition with that of the carboxyl group in the starting material.

Intramolecular Isotope Effects

Intramolecular isotope effects are the simplest to treat theoretically. Since the ground state for both isotopes is the same molecule, irrespective of which is undergoing reaction,

$$\sum_{i}^{3n-6} G(u_i)\,\Delta u_i = 0$$

An exact evaluation of the free energy function for the activated complex,

$$\sum_{i}^{3n'-6} G(u_i^{\ddagger})\,\Delta u_i^{\ddagger}$$

is not possible for complex reactions. Bigeleisen, however, has shown that it may be neglected, as a first approximation, since the values of the free energy functions for the first row elements of the periodic table are themselves small at room temperature(14). Physically this is equivalent to the assumption that the isotopic atoms are completely separated in the activated complex, i.e.,

$$\sum_{i}^{3n'-6} G(u_i^{\neq})\,\Delta u_i^{\neq} = 0$$

Therefore, in the special case of intramolecular bond rupture, the ratio of the rate constants is given by

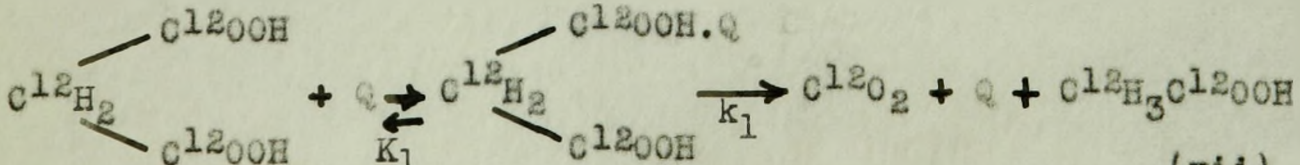
$$\frac{k_3}{k_2} = \left(\frac{m_3^*}{m_2^*}\right)^{\frac{1}{2}} \qquad (49)$$

The Bigeleisen equation, therefore, states that the intramolecular isotope effect depends only on the reduced mass of the bond being broken and is independent of temperature. For C-C bond rupture the calculated values of the C^{13} and C^{14} intramolecular isotope effects are 1.0198 and 1.0380, respectively. In other words the theory predicts that C^{12}-C^{12} bonds will rupture 2 per cent more frequently than C^{12}-C^{13} bonds, 4 per cent more frequently than C^{12}-C^{14} bonds, and that the ratio of the C^{14} to the C^{13} isotope effects should be 2.0.

In contrast to the erratic results of the C^{14} studies, the early experimental values for C^{13} intramolecular isotope effects were in reasonable agreement with the theoretical predictions. The results of representative studies are compared with the theoretical values in Table I. In these studies the isotope effects were determined for starting materials having normal isotopic abundances. Mass spectrometers were used in the measurement of all the isotopic ratios.

Yankwich and coworkers have investigated the C^{13} intramolecular isotope effects in the decarboxylation of malonic acid and the mono-anion of malonic acid in quinoline solution(21,22). In both cases the observed values of the intramolecular isotope effects were temperature dependent. The attempts that have been made to explain this discrepancy between the experimental results and Bigeleisen's simple bond rupture model are worth considering in some detail since they provide valuable information as to reaction mechanisms.

In a study of the decarboxylation of normal malonic acid in quinoline solution, Yankwich and Belford(21) found that impressed upon the unidirectional isotope effects were additional effects arising from one or more rapid reversible equilibria antecedent to the rate determining step. The reactions leading to products are

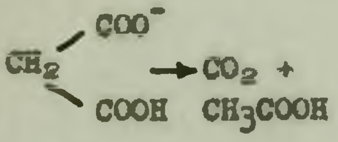
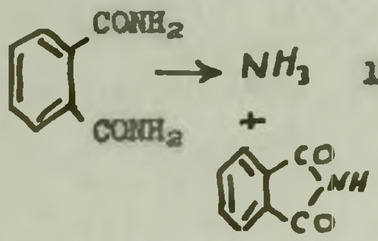


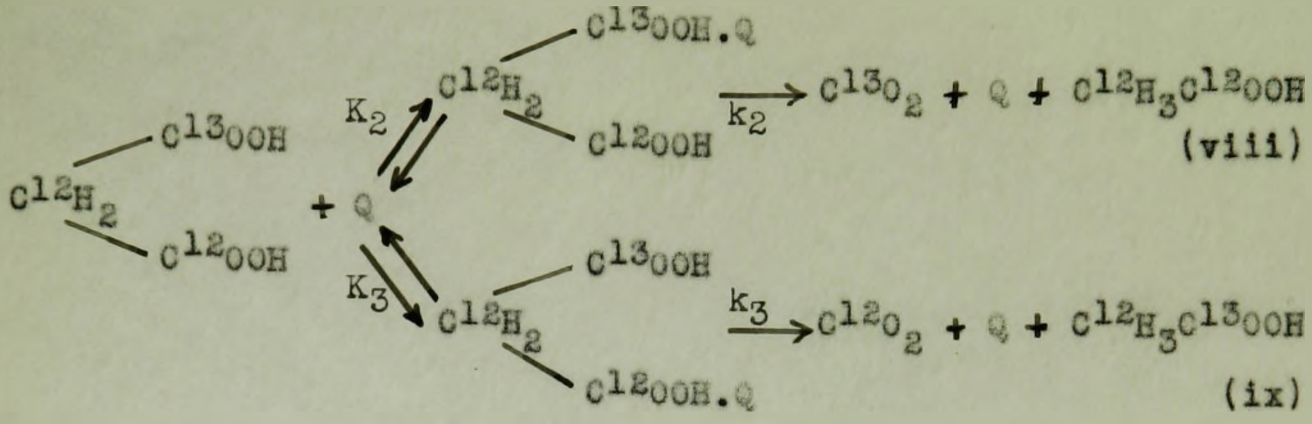
$$C^{12}H_2(C^{12}OOH)_2 + Q \underset{K_1}{\rightleftharpoons} C^{12}H_2(C^{12}OOH.Q)(C^{12}OOH) \xrightarrow[k_1]{} C^{12}O_2 + Q + C^{12}H_3C^{12}OOH$$

(vii)

TABLE I

ISOTOPE EFFECTS IN INTRAMOLECULAR BOND RUPTURE

System	Reaction Media	Isotopes compared	Temp. °C.	k_3/k_2		Reference
				Exp.	Theor.	
$CH_2(COOH)_2 \rightarrow CO_2 + CH_3COOH$	liquid	C^{12} vs C^{13}	137.5	1.020	1.0198	(4)
			138	1.026		(15)
			160	1.033		(16)
			138	1.026		(17)
			137	1.028		(18)
			140	1.0292		(19)
			140.5	1.0285		(20)
$CH_2(COOH)_2 \rightarrow CO_2 + CH_3COOH$	quinoline	C^{12} vs C^{13}	86	1.0445		(21)
			100	1.0410		(21)
			110	1.0373		(21)
			123	1.0356		(21)
			138	1.0317		(21)
	quinoline	C^{12} vs C^{13}	79	1.0354		(22)
			89.5	1.0368		(22)
			102.5	1.0352		(22)
			115.5	1.0332		(22)
			138	1.0328		(22)
$BrCH(COOH)_2 \rightarrow CO_2 + CH_3COOH$	liquid	C^{12} vs C^{13}	117	1.024		(18)
$NH_4NO_3 \rightarrow N_2O + H_2O$		O^{16} vs O^{18}	220	1.023	1.026	(23, 12)
	liquid	N^{14} vs N^{15}	180	1.014	1.016	(24, 25)



(viii)

(ix)

Where Q is the quinoline molecule, K is an equilibrium constant and k is the isotopic rate constant. An observed isotope effect then is a composite of the thermal rate constant ratio and the ratio of the corresponding equilibrium constants. Thus, the observed intramolecular isotope effect is given by

$$\left(\frac{k_3}{k_2}\right)_{obs.} = \frac{K_3}{K_2} \cdot \frac{k_3}{k_2} \qquad (51)$$

and the observed intermolecular isotope effect by

$$\left(\frac{k_3}{2k_2}\right)_{obs.} = \frac{K_1}{K_2} \cdot \frac{k_1}{2k_2} \qquad (52)$$

Yankwich and Belford calculated $k_1/2k_2$ for different temperatures using the simple bond rupture model of Bigeleisen and by substituting these values into equation (52) obtained the corresponding K_1/K_2 values. One would expect that $K_1 = K_3$ since both constants correspond to interaction of solvent molecules with a C^{12} carboxyl group, hence

$$\frac{K_1}{K_2} = \frac{K_3}{K_2} \tag{53}$$

Substitution of this value of K_3/K_2 into equation (51) gives values of k_3/k_2, the intramolecular isotope effect independent of solvent interactions, at different temperatures. Contrary to the predictions of the Bigeleisen treatment, this effect was found to be temperature dependent, decreasing in magnitude with increasing temperature. This discrepancy between the experimental results and the theoretical predictions may arise from either an inadequate knowledge of general solvent effects or a failure of the simple bond rupture model. While neither of these possibilities can definitely be precluded, it should be noted that the simple Bigeleisen model has been satisfactory in analagous reaction systems.

Yankwich and Weber (82) have studied the C^{13} intramolecular and intermolecular isotope effects in the decarboxylation of the mono-anion of malonic acid in quinoline solution. From a comparison of the experimental results with those calculated using the Bigeleisen simple bond rupture model, it was concluded that superimposed upon the fractionation arising from unidirectional bond cleavage were equilibrium effects involving interaction of quinoline with the carboxylate group and an isotopic discrimination in anion formation,

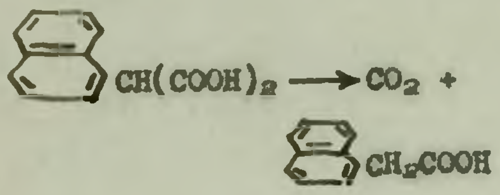
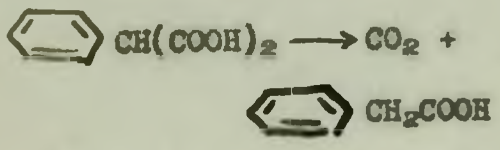
$$HOOC^{12}C^{12}H_2C^{13}OO^- \underset{}{\overset{K_x}{\rightleftarrows}} {}^-OOC^{12}C^{12}H_2C^{13}OOH \qquad (x)$$

Values obtained for K_x and K_1/K_2, where K_1 and K_2 are equilibrium constants for interaction of solvent with $-C^{12}OOH$ and $-C^{13}OOH$, respectively, were more consistent with the assumption that the carboxyl rather than the carboxylate ion was the source of the product carbon dioxide.

Until recently the C^{14} intramolecular isotope effects were anomalous with respect to theory. In general, the effects were considerably higher than the predicted value, 1.0380, and the agreement between the values reported by different workers was very poor. Moreover, in those cases where the C^{14} and C^{13} effects were determined in the same reaction system, the ratio of the C^{14} to the C^{13} effects was considerably greater than the value of 2.0 predicted by the Bigeleisen theory. The experimental values for C^{14} intramolecular isotope effects and the ratios of the C^{14} and C^{13} effects are listed in Table II.

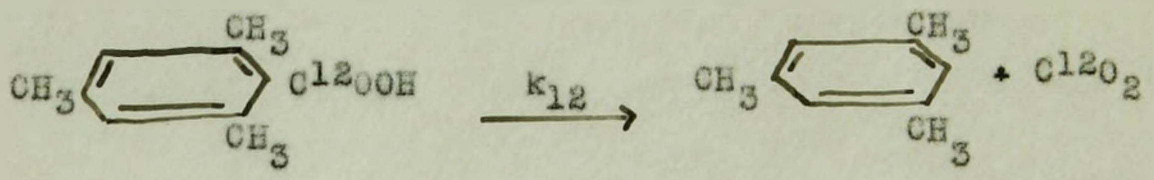
TABLE II

C^{14} ISOTOPE EFFECTS IN INTRAMOLECULAR BOND RUPTURE

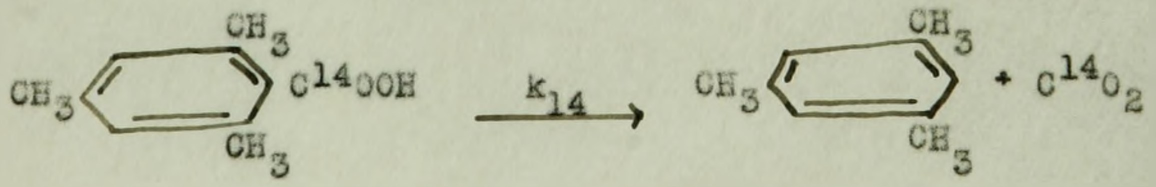
System	Reac'n Temp. °C.	k3/k2 (Exp) C14	k3/k2 (Exp) C13	100(k14/k12-1) / (k13/k12-1)	Ref.
$CH_2(COOH)_2 \longrightarrow CO_2 + CH_3COOH$	145	1.12			(3)
	153	1.06			(26)
	137	1.099	1.028	3.6	(18)
	138	1.100	1.026	3.8	(17)
	140.5	1.0545	1.0285	1.91	(20)
$BrCH(COOH)_2 \longrightarrow CO_2 + CH_3COOH$		1.41	1.12	3.4	(3)
	117	1.116	1.024	4.8	(18)
$(COOH)_2 \xrightarrow{H_2SO_4} CO_2 + CO + H_2O$	100	1.032			(5)
	80	1.067	1.033	2.0	(27)
	103	1.055	1.027	2.0	(27)
	73	1.098			(28)
	88	1.095			(28)
	165	1.076			(28)
	73	1.132			(28)
	163	1.088			(28)

In the majority of these investigations the C^{14}/C^{12} isotopic ratios were determined by counting techniques, the precision of which is considerably less than that possible with a mass spectrometer. There is no doubt that these counting techniques contributed to the anomalous results. Recent studies, however, suggest that contamination of the C^{14} labeled material may often result in serious experimental errors(29). Quantitative results are possible only if extreme precautions are taken to ensure the purity of all materials used in the study.

The importance of using a mass spectrometer for the measurement of the C^{12}/C^{14} isotopic ratios was first recognized by Stevens, Pepper and Lounsbury(30). These authors studied the C^{14} and C^{13} intermolecular isotope in the decarboxylation of carboxyl labeled mesitoic acid in sulphuric acid at 30°C., i.e.,



(xi)



(xii)

A mass spectrometer was used to measure both the C^{14} and the C^{13} isotopic ratios. They reported the values k_{12}/k_{14} = 1.101 and k_{12}/k_{13} = 1.038. The C^{13} effect is in good agreement with the C^{13} intermolecular isotope effects reported by other workers. However, the C^{14} effect is considerably higher than expected and the ratio of the C^{14} to the C^{13} effect, 2.7, is greater than the predicted value. It is difficult to see how contamination of materials could have occurred in this careful investigation. Nevertheless, the difficulty which other workers have had in obtaining reliable C^{14} results at least leaves open the possibility that this abnormally high C^{14} effect is incorrect. Further study of this system would be desirable.

Recently Yankwich, Promislow and Nystrom have published the results of a painstaking study of the intramolecular isotope effects in the decarboxylation of C^{13} and C^{14} carboxy labeled malonic acids (20). Extreme precautions were taken to avoid errors due to contamination. Both the C^{14} and C^{13} samples were analyzed in a mass spectrometer for the highest precision possible. The isotope effects observed for the C^{14} and C^{13} labeled acids were 1.0545 and 1.0285, respectively. The C^{14} effect is much closer to the value predicted by theory and the ratio of the

C^{14} effect to the C^{13} effect is 1.91, which is in good agreement with the theoretical value of 2.00. As in the case of the C^{13} intramolecular isotope effects, the best value for the C^{14} effect, 1.0545, is significantly higher than the theoretical value, 1.0380, calculated as the square root of the reduced mass (Equation 49).

The large discrepancy between the experimental C^{14} isotope effects and theoretical values, prior to the work of Yankwich, Promislov and Nystrom, prompted the speculation that perhaps the C^{13} effects rather than the C^{14} effects were anomalous. C^{13} has both a resultant nuclear spin and magnetic moment while the spins and moments of C^{12} and C^{14} are both zero. Recently, experiments have been designed to discover the effect of the inhomogeneous magnetic field resulting from such a magnetic moment on reaction rates and isotope effects. It has been argued by Pitzer and Gelles(31) that a paramagnetic ion such as dysprosium, at a distance of a few angstroms from a C-C bond, would cause an inhomogeneous field comparable to that caused by a C^{13} atom at one end of that bond.

Diamagnetic rare earth ions have practically no catalytic effect on the rate of decarboxylation of phenylmalonic acid but do catalyze the decarboxylation of oxalacetic acid somewhat. On the other hand, the paramagnetic ion Dy^{3+} increases the rate of decarboxylation of phenylmalonic and oxalacetic acids by 10 and 15 per cent, respectively, while Gd^{3+} accelerates the rate of decarboxylation of oxalacetic acid by 25 per cent (32).

Gelles and Reid (33) have studied the C^{13} isotope effect in the decarboxylation of normal oxalacetic acid, alone and in the presence of Y^{3+} and Dy^{3+}. It was found that diamagnetic ion Y^{3+} had no measurable influence on the isotope effect. However, when the strongly paramagnetic ion Dy^{3+} was added to the system, the C^{13} isotope effect increased from a value of six per cent, for the acid alone, to 10 per cent, that is, the Dy^{3+} ions catalyzed the fission of C^{12}-C^{12} bonds about four per cent more strongly than the fission of C^{12}-C^{13} bonds.

In the kinetic study it was found that Gd^{3+}, which has a larger number of unshared electrons than Dy^{3+}, was a better catalyst than Dy^{3+} (34). This suggests that the magnetic effect is a function of electronic spin properties rather than the total magnetic property of the ion. Gelles and Reid suggest that the C^{13} nuclear spin-electronic spin interactions are of the same order of magnitude as the electron spin interactions produced at a greater distance by a paramagnetic ion. Consequently, they postulate that the paramagnetic ion Dy^{3+} will have more influence on the C^{12}-C^{12} bond than on the C^{12}-C^{13} bond where the C^{13} nuclear spin effects are already operative. The authors propose to test this hypothesis by performing analagous experiments with C^{14}, which like C^{12} has zero nuclear spin. Further work on this interesting problem should contribute to our knowledge of the factors which influence isotope effects.

Intermolecular Isotope Effects in Bond Rupture Processes

The intermolecular isotope effect is defined as the relative rates with which molecules differing in the isotopic mass of a particular atom undergo chemical reaction, e.g., bond rupture. In decarboxylation studies the intermolecular effect is defined as k_1/nk_2 where n is the number of identical carboxyl groups in the parent molecule.

The theoretical treatment of intermolecular isotope effects in bond breaking reactions is more difficult than the case of intramolecular effects since all the terms in the Bigeleisen equation must be evaluated. For simple bond rupture the Bigeleisen theory predicts that the reacting molecule is more "tightly bound" in the initial state than in the activated complex, that is,

$$\sum_{i}^{3n-6} G(u_i)\,\Delta u_i > \sum_{i}^{3n'-6} G(u_i^{\neq})\,\Delta u_i^{\neq}$$

Consequently, the free energy term will be greater than unity and will act in the same direction as the effective mass term. Since both of these quantities are greater than unity and acting in the same direction, the overall isotope effect will be greater than the value of the effective mass term.

In the absence of specific knowledge of the activated complex, some reasonable model must be assumed before calculations can be made. The simplest model possible is to assume that the reaction can be treated as the decomposition of an imaginary diatomic molecule and that the frequencies of all bonds other than the one being ruptured are unchanged during the transition from the initial state to the activated complex. Since the bond is assumed to be completely broken in the activated complex

$$\sum_{i}^{3n'-6} G(u_i^{\neq})\,\Delta u_i^{\neq} = 0$$

Therefore, the ratio of rate constants is given by

$$\frac{k_1}{k_2} = \left(\frac{m_2^*}{m_1^*}\right)^{1/2} \left[1 + \sum_{i}^{3n-6} G(u_i)\,\Delta u_i\right] \qquad (48)$$

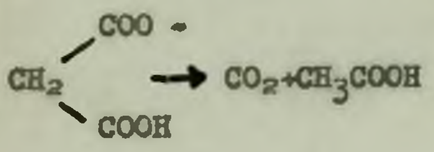
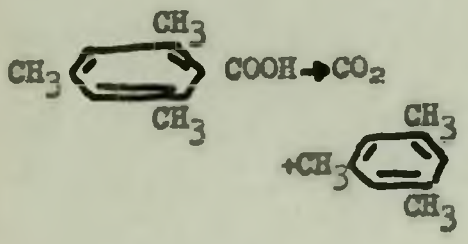
The value of k_1/k_2 can be calculated from equation (48) taking the effective mass term as the reduced mass of the atoms in the bond ruptured and evaluating $\sum_{i}^{3n-6} G(u_i)\Delta u_i$ from spectroscopic data. Isotope effects calculated in this way give an upper limit to the isotopic fractionation possible. The calculated effect will be too large to the extent that the bonds are not completely broken in the activated complex.

Further, the theory predicts that intermolecular isotope effects will be temperature dependent. The value of the free energy term varies with the reaction temperature since $u = hc\omega/kT$. As the temperature of the reaction system is increased, the contribution of the free energy term to the ratio of rate constants is reduced and k_1/k_2 approaches the value of the effective mass term as a lower limit.

Intermolecular isotope effects have been observed in a number of different reactions. However, reactions which proceed by a stepwise mechanism cannot be interpreted in terms of the simple bond rupture model of the Bigeleisen theory. Decarboxylation reactions approach most closely the simple bond rupture condition that must be satisfied before experimental results can be compared with the theoretical predictions. Experimental and theoretical values for C^{13} intermolecular isotope effects observed in decarboxylation reactions are tabulated in Table III.

TABLE III

C^{13} ISOTOPE EFFECTS IN INTERMOLECULAR BOND BREAKING REACTIONS

System	Media	Temp.	k_1/nk_2		Ref.
			Exp.	Theor.	
$CH_2(COOH)_2 \rightarrow CO_2 + CH_3COOH$	Liquid	138	1.037	1.035	(4, 14)
		138	1.046		(15, 14)
		137-196	1.036	1.035 - 1.031	(35, 14)
	80% H_2SO_4	56	1.0453	1.043	(36)
		79	1.0376	1.040	(36)
		100	1.0348	1.037	(36)
		129	1.0336	1.035	(36)
	Quinoline	34	1.0567	1.045	(37)
		59	1.0494	1.042	(37)
		79	1.0438	1.040	(37)
		99	1.0409	1.037	(37)
		118	1.0379	1.036	(37)
 $CH_2(COO^-)(COOH) \rightarrow CO_2 + CH_3COOH$	Quinoline	67.5	1.0417	1.041	(38)
		79	1.0415	1.040	(38)
		98	1.0391	1.037	(38)
		119	1.0377	1.036	(38)
	H_2SO_4	30	1.038		(30)
	86% H_2SO_4	61.2	1.0361	1.041	(39)
		92.0	1.0321	1.038	(39)
$Cl_3CCOO^- \rightarrow CO_2 + Cl_3CH$	H_2SO_4	70.4	1.0338		(40)

Lindsay, Bourns and Thode did not detect any temperature dependence of the intermolecular isotope effect in the decarboxylation of liquid malonic acid(35). The isotope effect $k_1/2k_2$ was calculated from the isotopic composition of the first few per cent carbon dioxide evolved and the carbon dioxide resulting from complete decarboxylation of the acid. Yankwich, Belford and Fraenkel(36) have pointed out that since the rates of decarboxylation and melting of malonic acid are comparable, much of the carbon dioxide evolved during the first few per cent reaction was formed at the melting temperature of the acid, and hence, the study did not permit an evaluation of the temperature dependence of the isotope effect. A temperature dependence has been observed by Yankwich and coworkers in the decarboxylation of malonic acid in sulphuric acid(36) and quinoline solution(37) and of the monoanion of malonic acid in quinoline solution(38); these values are given in Table III.

In the decarboxylation of normal malonic acid in sulphuric acid solution the C^{13} intermolecular isotope effects decreased as the reaction temperature was raised in accordance with the predictions of the Bigeleisen theory(36). The plot of the logarithm of the isotope effects against the reciprocal of the temperature, however, gave a curve instead of the straight line predicted by theory. The isotope effects at the higher temperatures were very close to the predicted values. The evidence indicated that the reactive species was the undissociated acid, but the data could not be interpreted by a single mechanism over the whole temperature range. The low temperature effect can be explained if one or more rapid reversible equilibria are presumed to operate prior to the rate determining step. While a catalytic decomposition may predominate in sulphuric acid at low temperatures, a thermal reaction is predominant at higher temperatures.

The observed C^{13} intermolecular isotope effects, $k_1/2k_2$ (see page 46), in the decarboxylation of normal malonic acid in quinoline solution departed significantly from the predictions of the Bigeleisen model for simple bond rupture(37). The divergence was greatest at the lower reaction temperatures studied. At higher temperatures, the observed isotope effects approached the theoretical values. The authors suggest that the experimental results can be explained if there are presumed to operate one or more rapid reversible equilibria antecedent to the rate determining step and involving interaction of the solvent molecules with the carboxylate group. Thus

$$\left(\frac{k_1}{2k_2}\right)_{\text{obs.}} = \left(\frac{K_1}{K_2}\right) \cdot \left(\frac{k_1}{2k_2}\right) \qquad (52)$$

where K_1/K_2 is greater than unity (see page 35). In contrast to the malonic acid-sulphuric acid system the results for the malonic acid-quinoline system can be explained by a single mechanism operating over the entire temperature range studied. The isotope effect results did not permit a choice between a solvation equilibrium followed by a thermal uncatalyzed decomposition of the complex and a solvation equilibrium followed by a bimolecular decomposition of the complex. The low entropy of activation for the reaction, however, would seem to require that the rate determining step be a bimolecular process involving a molecule of solvent.

Of particular interest insofar as the work of this thesis is concerned is the observation of Yankwich and Belford that the ratio $k_1/2k_3$, the relative rates of C^{12}-C^{12} bond rupture in unlabeled and labeled malonic acid, decreases with decreasing temperature and at approximately 90° becomes smaller than unity. In evaluating this ratio the assumption was made that the solvation equilibrium constants for solvation of C^{12} carboxyl groups in labeled and unlabeled acid molecules are equal (see page 35). Thus, $k_1/2k_3$ is given by

$$\frac{k_1}{2k_3} = \frac{(k_1/2k_2)\ \mathrm{obs}}{(k_2/\ k_3)\ \mathrm{obs}} . \qquad (54)$$

The authors suggest that this reverse temperature coefficient may result from an increase in the stretching vibration between the carbon atoms which remain joined. The consequence of this would be to give a free energy term for the activated complex which is greater than the corresponding term for the initial state and hence an overall free energy term which is smaller than unity and which would approach unity as an upper limit with increasing temperature. However, in a later publication Yankwich and Weber conclude that the temperature coefficient of $k_1/2k_3$ is too large to meet the conditions necessary for the value of the equilibrium constant of the isotope enchange reaction to cross unity(22). Consequently, the interpretation of the results for the decarboxylation of malonic acid in quinoline solution are still open to speculation.

Very few investigations have been concerned with C^{14} intermolecular isotope effects in simple bond breaking reactions. The most significant of these was the study of the C^{14} and C^{13} intermolecular isotope effects in the decarboxylation of mesitoic acid by Stevens, Pepper and Lounsbury(30). This investigation was discussed in the section describing intramolecular isotope effects (see page 39).

Bigeleisen and Wolfsberg have extended the theory of the relative rates of reaction of isotopic molecules to three center reaction systems where the problem of simultaneous bond rupture and bond formation is considered (12, 13). For the three atom reaction,

$$A + BC \rightarrow A\ldots B\ldots C \rightarrow AB + C \,, \qquad \text{(xii)}$$

in which the bond between atoms B and C is broken and the bond A-B formed, the reaction coordinate x_L may be written by the extension of a method developed by Slater for simple bond rupture as

$$x_L = \alpha |r_B - r_C| - \beta |r_B - r_A| \qquad (55)$$

where $r_B - r_C$ and $r_B - r_A$ are the separations of B - C and B - A, respectively, and β/α determines the relative amount of bond formation between A and B to bond stretching between B and C. Then, irrespective of the nature of the other coordinates, the reaction coordinate, x_L , leads to a frequency ν_L which depends on the masses of A, B and C alone. For the case of simultaneous bond rupture and bond formation the "effective mass" term has the form,

$$\nu_L \propto \left[\alpha^2 (m_B^{-1} + m_C^{-1}) + \beta^2 (m_B^{-1} + m_A^{-1}) + 2\alpha\beta\, m_B^{-1} \right]^{\frac{1}{2}}$$

and the ratio of the rate constants for isotopic molecules is given by

$$\frac{k_1}{k_2} = \frac{\kappa_1}{\kappa_2}\left[\frac{(m_{B_1}^{-1}+m_{C_1}^{-1}) + \rho(m_{B_1}^{-1}+ m_{A_1}^{-1}) + 2\rho^{\frac{1}{2}} m_{B_1}^{-1})}{(m_{B_2}^{-1}+m_{C_2}^{-1}) + \rho(m_{B_2}^{-1}+ m_{A_2}^{-1}) + 2\rho^{\frac{1}{2}} m_{B_2}^{-1}}\right]^{\frac{1}{2}} \frac{f}{f^{\neq}} \qquad (56)$$

where $\rho = \beta^2/\alpha^2$. When $\rho = 0$ equation (56) reduces to the simple case of bond rupture, equation (48).

Intermolecular Isotope Effects in Bond Formation Processes

The problem of intermolecular isotope effects in bond formation is analogous to the case of intermolecular isotope effects in bond rupture in that all the terms of the Bigeleisen equation must be evaluated before any exact calculations can be made. According to the Bigeleisen theory of simple bond formation the isotopic atoms associated with the new bond are more "tightly bound" in the activated complex than in the initial state, that is,

$$\sum^{3n'-6} G(u_i^{\neq})\,\Delta u_i^{\neq} > \sum^{3n-6} G(u_i)\,\Delta u_i$$

Therefore, the square bracket term of the Bigeleisen equation will be less than unity and tends to cancel the effective mass term. Since these terms are operating against each other, the theory predicts that the ratio of isotopic rate constants will have some value less than that of the effective mass term.

The theory is unable to predict which isotope will be favored in a unidirectional bond formation process since the value of k_1/k_2 will depend primarily on the magnitude of the square bracket term. Theoretically, there are three possibilities. The light isotope will have the greater rate constant, i.e. $k_1/k_2 > 1$, if the square bracket term is less than the effective mass term. If the two terms of the Bigeleisen equation just cancel each other, $k_1/k_2 = 1$, and there will be no fractionation. Finally, the heavy isotope will be favored, $k_1/k_2 < 1$, if the square bracket term is greater than the effective mass term.

The Bigeleisen equation must be simplified before any calculations can be made. A reasonable simplification is to assume that a bond formation process can be treated as the formation of a hypothetical bond from separated atoms and that the vibrational frequencies of all bonds, other than the one being formed, are unchanged on going from the initial state to the activated complex. Since the isotopic atoms are assumed to be an infinite distance apart in the initial state,

$$\sum_i^{3n-6} G(u_i)\ \Delta u_i = 0$$

and the ratio of the rate constants of the isotopic species is given by

$$\frac{k_1}{k_2} = \left(\frac{m_2^*}{m_1^*}\right)^{1/2} \left[1 - \sum_i^{3n\pm6} G(u_i^{\pm})\ \Delta u_i^{\pm}\right] \qquad (50)$$

In the absence of information concerning the exact nature of the activated complex, a model of the activated complex must be assumed before the free energy term, $\sum_{i}^{3n-6} G(u_i^{\ddagger})\Delta u_i^{\ddagger}$ can be evaluated from spectroscopic data. The assumption that the bond is completely formed in the activated complex is the simplest model possible. Using this model the free energy term can be evaluated directly from spectroscopic data. This estimate of k_1/k_2 will be less than the true value to the extent that the bond is not completely formed in the activated complex.

A further prediction of the simple Biegeleisen model is that the isotope effects occuring in bond formation reactions will be temperature dependent. Since the free energy function is defined in terms of $u = hc\omega/kT$, the exact value of both $G(u_i^{\ddagger})$ and $\Delta u_i^{\ddagger}$ will vary with the reaction temperature. Relatively large changes in the value of $\Delta u_i^{\ddagger}$, a small number, result from small temperature changes. As the reaction temperature is raised the values of both $G(u_i^{\ddagger})$ and $\Delta u_i^{\ddagger}$ decrease and the square bracket term tends to unity. Consequently, at higher temperatures there is less cancellation of the effective mass term and the value of k_1/k_2 tends to that of the effective mass term as an upper limit; in other words, the light isotope is favored by an increase in the reaction temperature.

In the above discussion it is assumed that the effective mass term in the Bigeleisen equation can be treated as the square root of the ratio of the reduced masses of the hypothetical diatomic molecules formed by reaction between isotopic atoms, a simplification which Slater has shown to be valid in the case of bond rupture(11). Bigeleisen has used this procedure to evaluate isotope effects in bond formation processes(6, 25). Indeed, the model developed by Wolfsberg and Bigeleisen to quantitatively describe the case of simultaneous bond rupture and bond formation is based on an extension of Slater's theorem(12). When a bond is formed by the reaction of separate molecules in solution, however, it is conceivable that the masses of the whole molecules should be used in the calculation of the effective mass term instead of just the atoms that form the bond. The value of the effective mass term calculated as the reduced mass of the molecules involved in the bond formation process, is considerably smaller than that based upon the simple diatomic model. This interpretation suggests that the isotope effect should vary with the molecular weights of the reacting species. On the other hand, the Bigeleisen model predicts that the isotope effect will be approximately the same for all reactions of similar type studied under comparable conditions. In both of these models it is assumed that the free energy terms are essentially the same throughout an homologous series of reactions. In fact, this assumption must be made before calculations based on the Bigeleisen model are possible, since spectroscopic data is available for only a few compounds. This assumption, however, may not be justified.

The problem of isotope effects in unidirectional bond formation processes has received little experimental attention to date. In fact, only one study of the isotope effect in a one-step bond formation reaction, which was essentially unidirectional in character, has been reported. Sheppard, Bader and Bourns observed very small effects in the bisulphite addition reaction of aldehydes and ketones at 25°C(8). Their results are tabulated in Table IV.

TABLE IV

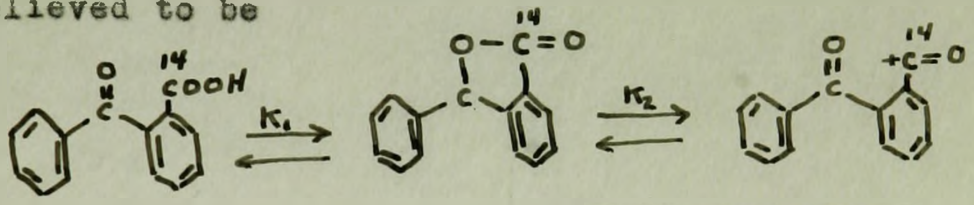
S^{34} ISOTOPE EFFECTS IN THE BISULPHITE ADDITION REACTION OF ALDEHYDES AND KETONES(6)

	k_{32}/k_{34}	
Carbonyl Compound	Exp.	Theor.
Heptaldehyde	1.001	1.001
2- Heptanone	0.999	
Benzaldehyde	1.003	
Anisaldehyde	0.998	

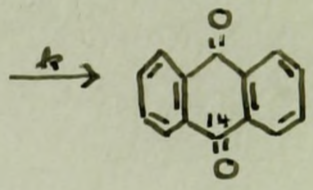
Since the bisulphite addition reaction is readily reversible and isotopic equilibrium between the bisulphite ion and the addition product is rapidly established, strictly unidirectional conditions could not be attained. The contribution of the reverse reaction was minimized by removing the precipitated product as rapidly as possible from the reaction system. The specific rate constant ratios, k_{32}/k_{34}, for the addition reaction were determined by measuring the fractionation after varying short reaction periods and extrapolating to zero time. A theoretical value of 1.001 for the k_{32}/k_{34} ratio was calculated from the simple Bigeleisen model on the assumptions that the effective mass term could be treated as the reduced mass of an hypothetical C-S molecule formed from separated atoms and that the new bond was completely formed in the activated complex. This result was in reasonable agreement with the experimental values.

In addition to the above investigation, a number of C^{14} isotope effects observed in certain complex reactions have been attributed to bond formation processes. However, the significance of many of these studies is doubtful since the reactions are known to proceed by equilibrium steps and in some reactions the rate determining step or steps are not specifically known. Moreover, as noted previously, contamination is a much more serious problem when labeled compounds are used(29). Two recent C^{14} investigation do deserve mention.

Stevens and Crowder(41) reported a value of k_{12}/k_{14} = 0.931 in the condensation of benzoylbenzoic acid-carboxyl-1-C^{14} to anthraquinone-9-C^{14}. The mechanism of this condensation is believed to be



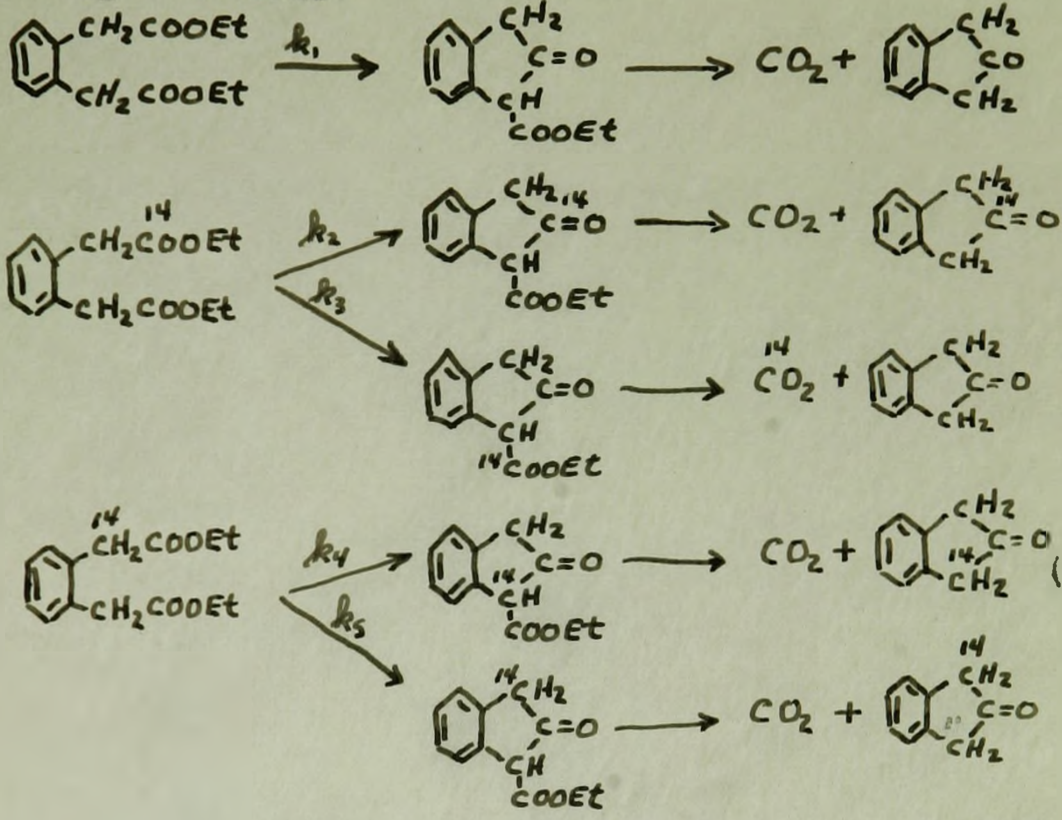
(xiii)



(xiv)

They attributed this "reverse isotope effect" to the formation of the C^{12} - C^{14} bond in the last step of the condensation. Ropp has reexamined the isotope effect in this condensation and found a normal effect of $k^{12}/ k^{14} = 1.03$(29). He attributed the large "reverse" effect, reported by Stevens and Crowder, to isotopic dilution of the product in the steps following the synthesis of the acid.

Recently Carrick and Fry have reported C^{14} isotope effects in the Dieckmann condensation of diethylphenylenediacetate-1-C^{14} and diethylphenylenediacetate-2-C^{14}(42). The isotope effects possible in this condensation arise from the following reactions:



(xv)

(xvi)

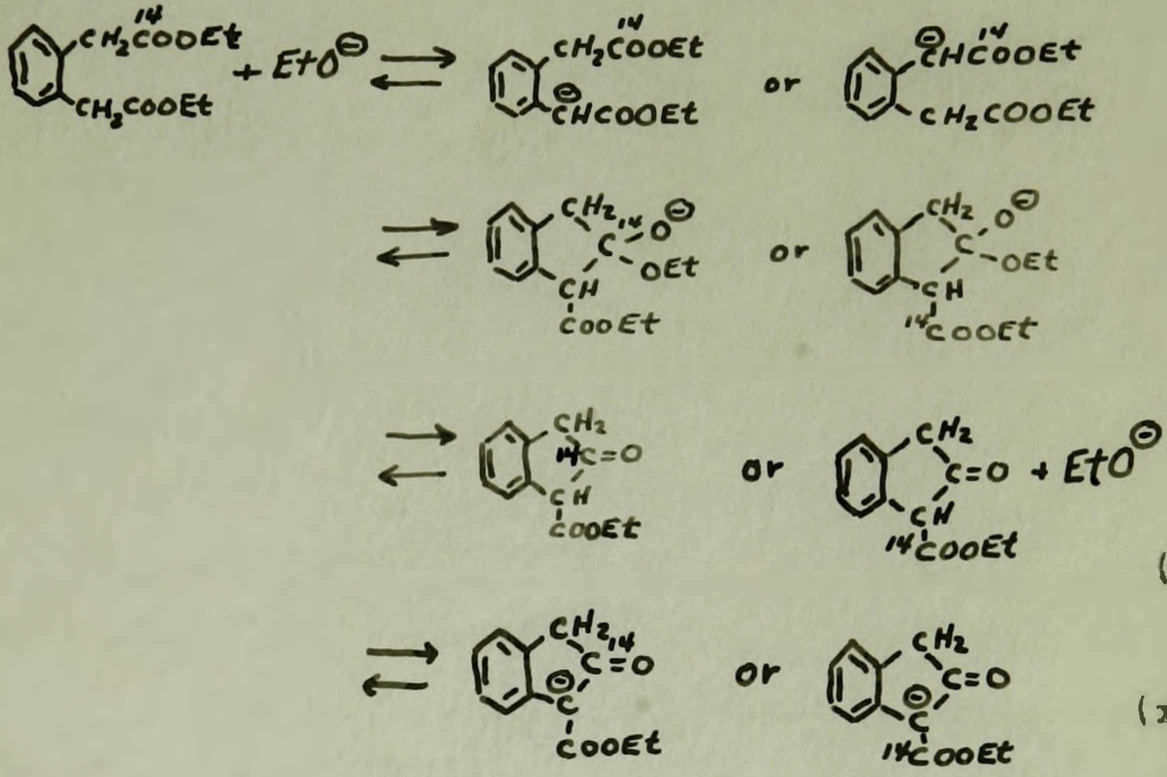
(xvii)

(xviii)

(xix)

In the cyclization of diethylphenylenediacetate-1-C^{14} intramolecular isotope effects, k_3/k_2, of 1.016 and 1.056 were observed in the solvents toluene and ethanol, respectively. Two intermolecular isotope effects were measured. The value of $k_1/2k_2$ in the cyclization of diethylphenylenediacetate-1-C^{14} was 1.084, while a value of 1.087 for $k_1/2k_4$ was observed in the condensation of diethylphenylenediacetate-2-C^{14}. Both reactions were carried out in ethanol.

The accepted mechanism for the condensation of diethylphenylenediacetate-1-C^{14} may be represented as follows:



(xx)

(xxi)

(xxii)

(xxiii)

A similar mechanism can be written for the condensation of diethylphenylenediacetate-2-C^{14}. The condensation was made effectively irreversible by using excess base.

The authors argue that the large isotope effect in the condensation of diethylphenylenediacetate-1-C^{14} in ethanol is evidence that neither equations (xx) or (xxiii) represent the rate determining step since the isotopic center is not involved in either step. For diethylphenylenediacetate-1-C^{14} either steps (xxi) or (xxii) can be rate determining. A C-C bond is formed in (xxi) and a C-O bond is ruptured in (xxii). A large isotope effect was also observed in the reaction of diethylphenylenediacetate-2-C^{14}. Consequently, it is reasoned that equation (xxii) cannot represent the rate controlling step since this step does not involve the isotopic center. The only step which involves the isotopic center in both labeled esters is (xxi). Therefore the authors conclude that the rate controlling step is the formation of the C-C bond.

In this study the isotopic concentrations were measured by counting techniques. Yankwich et al (20) and Ropp (29) have shown that elaborate precautions are necessary when working with C^{14}. Even a slight contamination can result in serious errors. It is possible that the experimental difficulties were not fully appreciated in this investigation. Further, it is doubtful if the isotope effects observed in complex reactions, such as the Dieckmann condensation of diethylphenylenediacetate, can be interpreted in terms of simple bond formation or rupture. In such reactions it has generally been found that the observed effect is a composite of equilibrium and bond rupture or formation contributions. The large isotope effect attributed to a bond formation process by Carrick and Fry is anomalous and warrants further investigation.

Unidirectional Bond Formation Reactions

The addition reaction of alkylamines with alkyl halides

$$R_3N + R'X \longrightarrow [R'R_3N]^{+}\overset{-}{X} \qquad (1)$$

is ideal for the investigation of the isotope effects in unidirectional bond formation processes. The nitrogen isotope effect can be readily measured since only bond formation occurs with respect to the nitrogen isotopes.

The reaction of amines with halides was first studied by Menschutkin and found to be bimolecular(43). These reactions have been studied intensively by many workers who were primarily interested in relating the observed kinetic data with the factors of the Arrhenius equation. Consequently, rate constants and activation energies are available for a large number of amine-halide-solvent systems.

The primary objective of this investigation was to determine the magnitude and temperature dependence of the isotope effects in one-step bond formation reactions and to compare these experimental values with the predictions of the simple bond formation model of the Bigeleisen theory. A secondary objective was to discover if there is any correlation between the observed isotope effects and activation energies. Therefore, reactions with marked differences in activation energies were included in this study. The activation energies of the reactions in which isotope effects were measured are listed in Table V.

TABLE V

ACTIVATION ENERGIES OF AMINE-HALIDE REACTIONS IN BENZENE

System	Act. Energy cal./mole	Reference
$(C_2H_5)_3N + CH_3I$	9,700	(44)
	9,300	(45)
	9,650	(46)
$(C_2H_5)_3N + C_2H_5I$	11,400	(47)
$(C_2H_5)_3N + (CH_3)_2CHI$	17,100	(44)
	16,000	(46)

EXPERIMENTAL

Introduction

Alkyl amines react with alkyl halides to give quaternary ammonium salts,

$$R_3N + R'X \longrightarrow [R_3R'N]^+X^- \qquad (1)$$

Since many of these reactions can be made to proceed irreversibly under ordinary reaction conditions and, with respect to the nitrogen atom, are simple bond formation processes, they are very convenient systems for the study of bond formation isotope effects.

An intermolecular isotope effect, expressed as the ratio of the reaction rates for light and heavy isotopic molecules, k_L/k_H, is given by the ratio of the isotopes for the atom in question in the product formed when the reaction has proceeded to an infinitesimally small extent, and in the initial reactant or the product of complete reaction. In practice, the isotopic ratio is determined for the product formed after some known extent of reaction and the true isotope effect is then calculated using an equation given by Stevens(48). Since in the present investigation the isotope effects were very small, the ratio of the nitrogen isotopes in the product of 2 - 10 per cent reaction was the same as that of the product corresponding to infinitesimal reaction within the precision of the measurements.

The ratio of the nitrogen isotopes was determined by a mass spectrometric analysis of molecular nitrogen formed from the quaternary salt. The product of complete reaction was used in preference to the original amine since this would ensure that the nitrogen samples, whose isotopic ratios were being compared, had been prepared under comparable conditions.

The composition of the nitrogen isotopes in a quaternary salt is given by:

$$\frac{(R_4N^{14})^+}{(R_4N^{15})^+} = \frac{2\,(N^{14}N^{14})}{(N^{14}N^{15})} \qquad (57)$$

Therefore, the isotope effect may be calculated from

$$\frac{k^{14}}{k^{15}} = \frac{(N_2^{28}/N_2^{29})_{\text{partial reaction}}}{(N_2^{28}/N_2^{29})_{\text{complete reaction}}} \qquad (58)$$

where the isotopic ratios are normalized values.

Isotope effects were determined for the following reaction systems:

Triethylamine and methyl iodide:-

$$(C_2H_5)_3N + CH_3I \longrightarrow [CH_3(C_2H_5)_3N]^+I^- \qquad (xxiv)$$

Since this is a rather fast reaction the isotope effect was measured at only one temperature, 8°C.

Triethylamine and ethyl iodide:-

$$(C_2H_5)_3N + C_2H_5I \longrightarrow \left[(C_2H_5)_4N\right]^+ I^- . \qquad (xxv)$$

Isotope effects were measured at the reaction temperatures, 6, 25, 56 and 78°C.

Triethylamine and isopropyl iodide:-

$$(C_2H_5)_3N + (CH_3)_2CHI \longrightarrow \left[\left\{(CH_3)_2CH\right\}(C_2H_5)_3N\right]^+ I^- . \qquad (xxvi)$$

An attempt was made to measure the isotope effect in this reaction at 61°C. As this reaction is very slow at moderate temperatures, no attempt was made to prepare the product of complete reaction. Instead, the partially reacted isopropyltriethylammonium iodide was prepared from the same stock of amine as the tetraethylammonium iodide and the isotope effect was calculated by relating the isotopic ratio in the product of partial reaction to that of tetraethylammonium iodide formed by complete reaction of the amine with ethyl iodide.

Trimethylamine and ethyl bromide:-

$$(CH_3)_3N + C_2H_5Br \longrightarrow \left[C_2H_5\,(CH_3)_3N\right]^+ \bar{B}r \; . \qquad (xxvii)$$

Isotope effect measurements were made at 6° and 25°C for this reaction.

All the quaternary salt formation reactions were carried out in thiophene-free, anhydrous benzene solution. This solvent was selected because it had a number of significant advantages. Previous kinetic work had shown that the reactions between alkyl amines and halides are unidirectional in benzene, an important consideration, since many amine-halide addition reactions are known to be equilibrium processes. Furthermore, rate constants and activation energies have been determined for many reaction systems in this solvent. Finally, it was anticipated that the quaternary ammonium salts would precipitate quantitatively from a benzene solution and that the salts could be easily purified.

The partial reactions were carried out at a constant temperature, the product was filtered quickly, washed and dried. Since the quaternary salts were found to be insoluble in each of the components of the reaction mixture, i.e. benzene, amine and halide, the per cent reaction was determined by comparing the weight of the product formed during the reaction period with that calculated for complete reaction.

Since it is known that some quaternary salt formation reactions are equilibrium processes, it was necessary to demonstrate conclusively that the reactions included in this study were truly unidirectional under the conditions used. Large quantities of unreacted amines and alkyl halides are present in the preparation of the partially reacted quaternary ammonium salts. Since amines are nucleophilic compounds, it is conceivable that this unreacted amine could attack the quaternary salt in either a displacement reaction.

$$\left[R_3NCH_2CH_2R'\right]^+ X^- + R''_3N \longrightarrow \left[R''_3N\ CH_2CH_2R'\right]^+X^- + R_3N \quad ,(xxviii)$$

or an elimination reaction

$$\left[R_3NCH_2CH_2R'\right]^+X^- + R''_3N \longrightarrow CH_2 = CHR' + \left[R''_3NH\right]^+X^- + R_3N. \quad (xxix)$$

Since a C-N bond in the quaternary ammonium salt is broken in either case, it is possible that the bond formation isotope effect would be masked by that due to bond rupture, a process which would be expected to favor the N^{15} isotope. If either reaction occurs the system would not be unidirectional and it would be impossible to measure the isotope effect associated with bond formation only. Consequently, three of the bond formation reactions included in this study were examined for each of the above side reactions.

In the first test for the possible side reactions of elimination and displacement, tetraethylammonium iodide was stirred in a benzene solution containing a large amount of trimethylamine for a period considerably longer than that required for partial reaction. The quaternary salt recovered was recrystallized from ethanol, the per cent nitrogen was determined by Kjeldahl digestion and these values were compared with the recoveries from pure tetraethylammonium iodide. If either displacement or elimination had occurred to any appreciable extent during the equilibration period, the per cent nitrogen recovered would have been quite different from that calculated for the starting material. Ethyltrimethylammonium iodide, a product of a displacement reaction, would give ammonia recoveries considerably higher than calculated for pure tetraethylammonium iodide. Since the nitrogen recovered from the equilibrated material corresponded closely to that obtained from the pure starting material, it was concluded that there was little, if any, attack of trimethylamine on the quaternary salt.

In a similar experiment ethyltrimethylammonium bromide was equilibrated in a solution composed of triethylamine and benzene. Again, the nitrogen determined by Kjeldahl digestion of the recovered material was identical with that found for pure ethyltrimethylammonium bromide indicating little, if any, attack during the equilibration period.

In contrast to these results, equilibration under reaction conditions of triethylisopropylammonium iodide with triethylamine furnished a material giving nitrogen Kjeldahl values which were considerably higher than those obtained for the pure salt. Furthermore, it was noted that gas was evolved during the equilibration and that the recovered material possessed solubility properties which differed from those of the starting material. These results indicate that this quaternary salt underwent partial elimination under the equilibration conditions (and hence, under the conditions used in salt formation) to form propylene and triethylammonium iodide, the latter being responsible for the high Kjeldahl values. Further evidence that elimination can occur under the reaction conditions was obtained when triethylisopropylammonium iodide was heated in benzene solution with triethylamine of different isotopic abundance. It was found that the isotopic abundance of the salt had shifted towards that of the amine. This result can be understood if the product recovered after the equilibration was contaminated with triethylammonium iodide formed by the elimination reaction. The occurrence of elimination explains the observation that the quaternary salt formed by the partial reaction of triethylamine with isopropyl iodide was significantly enriched in N^{15} (see page 146). The elimination reaction on the first formed product would favor $C-N^{14}$ bond rupture and the remaining undecomposed salt would, therefore, be enriched in the heavier isotope.

The experimental procedures and detailed results for these equilibrations will be presented later in this section.

The relative abundances of the nitrogen isotopes in the quaternary ammonium salts produced in the bond formation reactions studied were determined by mass spectrometer analyses on molecular nitrogen. The nitrogen in an organic compound can be degraded directly to molecular nitrogen by a Dumas combustion or indirectly by a Kjeldahl digestion. In the Dumas procedure the nitrogen compound is oxidized at high temperatures over a copper oxide catalyst and the nitrogen is collected over a concentrated solution of potassium hydroxide which absorbs the carbon dioxide produced during the combustion. In the Kjeldahl procedure the nitrogen compound is degraded to ammonia, and subsequently, is oxidized to nitrogen with sodium hyprobromite in a high vacuum system.

The degradation procedure used to obtain molecular nitrogen must satisfy two major requirements. Each step in the degradation must proceed quantitatively and the nitrogen produced must be of high purity. Incomplete conversion in any of the degradation steps could result in an isotopic fractionation not due solely to the reaction under investigation. If each reaction in the degradation scheme proceeds quantitatively, an error of this kind is impossible. The nitrogen sample must be free of contaminants, especially those which give rise to mass ions in the nitrogen mass range. Since the 29 peak, $(N^{14}-N^{15})^+$, is small, any impurity which contributes to the 29 peak would make the accurate measurement of this mass impossible. In any degradation procedure amines, hydrocarbons, carbon dioxide, carbon monoxide and nitrogen oxides are all possible contaminants, e.g., methyl amine, mass 31, is easily stripped of hydrogen atoms and thus could give rise to dissociation fragments in the nitrogen mass range. Since pure nitrogen was absolutely essential to the success of this investigation, each step in the degradation procedure was carefully examined.

When this study was initiated the Dumas method did not seem capable of giving nitrogen samples of the required purity. Nitrogen prepared by the Dumas procedure has been reported to contain contaminants which result from incomplete combustion of the starting materials and from gases released by the concentrated potassium hydroxide solution over which the nitrogen is collected*. On the other hand, Rittenberg has prepared nitrogen of high purity by first degrading the organic material to ammonia and then oxidizing the ammonia to nitrogen with sodium hypobromite(49). Since the Rittenberg procedure seemed the most promising, a search was made for a Kjeldahl procedure which would accomplish a quantitative degradation of quaternary ammonium salts to ammonia. It should be noted that quantitative conversion to titratable nitrogen does not necessarily mean a quantitative conversion to ammonia since amines behave as ammonia in acid-base titrations.

In initial experiments, the Kjeldahl digestion of tetramethylammonium iodide was studied using the mercuric

---

* Since this study was initiated, Dumas procedures, for the preparation of pure nitrogen for mass spectrometric analysis have been described by Holt and Hughes(50, 51), Ory, Prescott and Lyman(52) and Jones and Trenner(53).

oxide-potassium sulphate-sulphuric acid digestion mixture recommended by Willits, Coe and Ogg(54) and Willits and Ogg(55). Although good recoveries of ammonicial material resulted from short digestions of the iodide, the molecular nitrogen from these samples was contaminated. The distribution of the ion fragments suggested that both methylamine and ethylamine were present. When longer digestions were attempted, the recovery of ammoniacal material was poor.

It was suspected that iodine, liberated during the digestion, was oxidizing the ammonia to nitrogen. This surmise was tested by adding iodine to Kjeldahl digestions of triethylamine. In the absence of iodine the recovery of ammonia from the amine was high for long digestion periods. However, when iodine was added to the digestion mixture, the recovery of ammonia dropped to 50 per cent or less. This demonstrated that the iodide ion had to be removed from the quaternary compound before long digestion periods were possible. The interfering iodide ions were removed by reacting the quaternary iodide with an excess of silver hydroxide in water solution,

$$[(C_2H_5)_4N]^+I^- + AgOH \rightarrow [(C_2H_5)_4N]\overset{+}{O}H^- + AgI \ . \qquad (xxx)$$

The quaternary ammonium hydroxide was separated from the silver iodide and unreacted silver hydroxide by filtration. Titration of the quaternary ammonium hydroxide solutions with standard acid demonstrated that the iodide was quantitatively converted to the hydroxide. Since most alkyl quaternary ammonium hydroxides exist as hydrates which are stable only in solution, any attempt to isolate the quaternary hydroxide as such would have resulted in some decomposition and loss of amine(56, 57). Therefore, the hydroxide was converted quantitatively to the stable quaternary sulphate. Prior to digestion the quaternary sulphate was isolated by a vacuum distillation at moderate temperatures.

Before nitrogen samples were prepared for isotopic analyses the effect of digestion time on ammonia recovery and the purity of the samples prepared by subsequent hypobromite oxidation was investigated. It was found that recoveries decreased only slightly as the digestion period was increased from six to twenty-four hours, but were significantly lower for forty-eight hour digestions. Nitrogen samples prepared from tetraethylammonium sulphate digested for six, twelve, twenty-four and forty-eight hours were examined in the mass spectrometer for contaminants and in all cases were found to be very pure. Only trace quantities of the contaminants, carbon dioxide, argon, potassium, oxygen and nitric oxide, were detected. On the basis of these results a twelve hour digestion period with the mercuric oxide-potassium sulphate-sulphuric acid Kjeldahl mixture was used in the Kjeldahl digestion of the quaternary ammonium salts prepared in this isotope effect study.

Finally, the ammonia was oxidized to nitrogen with sodium hypobromite in a high vacuum system,

$$2NH_3 + 3NaOBr \longrightarrow N_2 + 3NaBr + 3H_2O \ . \qquad \text{(xxxi)}$$

The extent to which the reaction was quantitative was investigated. The vacuum line was calibrated and the procedure standardized by oxidizing known quantities of pure ammonium chloride. The oxidation of both ammonium chloride and the ammoniacal material recovered from the Kjeldahl digestion was found to be quantitative.

The nitrogen samples were analyzed in a 180° direction focusing mass spectrometer(58). Since nitrogen is ionized to the N_2^+ ion in the spectrometer, the ratio of N^{14} to N^{15} is determined by measuring the relative abundances of the ions $(N^{14}-N^{14})^+$ and $(N^{14}-N^{15})^+$. Every effort was made to obtain as precise mass spectrometer measurements as possible.

The isotope effects were determined by two methods. First, the isotopic ratio of each sample was measured relative to that of a large reservoir of purified nitrogen, designated as tank nitrogen, taken as a reference standard to compensate for day to day variations in the spectrometer. An analysis consisted of the sequence, tank-sample-tank-sample, etc. performed as rapidly as possible. A sample analysis was accepted only if the data successfully met prescribed statistical tests. Next 95 per cent confidence intervals, relative to tank nitrogen, were computed for each sample. The isotope effect was then calculated by normalizing the difference between the 95 per cent confidence intervals for complete and partial reactions. Isotope effects were also obtained by intercomparing the samples themselves. A typical intercomparison consisted of the analytical sequence, complete-partial-complete-partial, etc. Samples prepared by partial reaction at different temperatures were also intercompared by the same procedure giving a ratio of the isotope effects for the two reaction temperatures employed. The intercomparison of partially and completely reacted products gives the isotope effect directly. Again 95 per cent confidence intervals were computed for each intercomparison which met the statistical requirements.

Experimental Procedure

Materials

Amine:- Triethylamine, British Drug House, Analar grade, and trimethylamine, Eastman Kodak, white label, were dried over potassium hydroxide and distilled prior to use. Triethylamine was collected over the temperature range 88.6-88.7°C and trimethylamine over the range 3.2-3.4°C. The amines were stored in a refrigerator at all times. Amine used in the preparation of a given series of quaternary salts was drawn from the same reservoir of purified material.

Alkyl Halides:- Eastman Kodak white label methyl iodide, ethyl iodide and ethyl bromide were dried over anhydrous calcium chloride and distilled rapidly at atmospheric pressure. The boiling ranges of the halides collected were methyl iodide, 42.2-42.3°C, ethyl iodide, 72.3-72.8°C, and ethyl bromide, 38.6-39.0 °C.

The isopropyl iodide was prepared by reacting red phosphorous and iodine with isopropanol according to the procedure described by Vogel(59). The iodide was purified by a vacuum distillation, b.p. 41°C. at 13.5 mm. All purified halides were stored in the refrigerator.

Solvent Benzene:- Commercial benzene, of low thiophene content, was treated with mercuric oxide and acetic acid, as described by Vogel(60), to remove the last traces of thiophene. The fraction boiling at 78°C was collected and stored over sodium.

Silver Hydroxide:- Silver hydroxide was prepared by treating a 20 per cent solution of analytical grade silver nitrate with a 10 per cent solution of sodium hydroxide in slight excess. The silver hydroxide was collected in a sintered glass funnel, washed with water until free of nitrate ion and dried at 78°C. The dry material was ground and stored in an amber bottle.

Kjeldahl Reagents:- Baker Analar grade mercuric oxide and potassium sulfate and Canadian Industries Limited C.P. sulphuric acid were used in the Kjeldahl digestions.

Sodium Hypobromite:- Sodium hypobromite was prepared following the procedure described by Rittenberg *et al*(49, 50). Sodium hydroxide, 200 gm., was dissolved in 300 ml water. To one-half of the above solution, cooled in an ice bath, 60 ml of bromine was added with vigorous stirring over a period of 30 minutes. The remainder of the sodium hydroxide solution was added and the resulting sodium hypobromite solution was placed in a refrigerator for a few days. This is necessary since freshly prepared sodium hypobromite tends to evolve oxygen. Finally, the precipitate of sodium bromide was removed by filtration and the solution was stored in a refrigerator until required.

Preparation Of Quaternary Salt Samples

Complete Reactions:- The reactions in which amines were converted quantitatively to quaternary salts were carried out in sealed flasks using an excess of halide. Reaction times and temperatures were such that complete conversion to product was ensured.

Since the reaction of triethylamine and isopropyl iodide is very slow, no attempt was made to force this reaction to completion. The isopropyltriethylammonium iodide formed by partial reaction was prepared from the same stock of triethylamine used in the triethylamine-ethyl iodide reaction. The isotope effect in this case was obtained by comparing the nitrogen isotopic abundance in the product of the partial reaction of triethylamine and isopropyl iodide with that from tetraethylammonium iodide formed by complete reaction.

The experimental conditions under which the completely reacted quaternary ammonium salts were prepared are given in TABLE VI.

Partial Reactions:- The reaction temperatures in the preparation of the partially reacted quaternary salts were controlled to ± 0.1°C. The benzene solutions of the reactants were brought to thermal equilibrium before being mixed. The amine-halide mixtures were stirred during the reaction period. When the reaction had proceeded to the desired extent, the quaternary salt was filtered as quickly as possible through a weighed sintered-glass filter. After filtration the precipitates were washed with benzene to remove unreacted amine and halide, dried under vacuum over phosphorous pentoxide and wax chips and weighed.

The experimental conditions under which the partially reacted quaternary ammonium salts were prepared are tabulated in TABLE VI.

TABLE VI

EXPERIMENTAL CONDITIONS FOR AMINE-HALIDE REACTIONS

Reaction	Run No.	Amine (moles)	Halide (moles)	Benzene (mls)	Reaction Temp.(°C)	Reaction Time (Hrs)	Percent Reac'n*
Et_3N+MeI	A1	0.036	0.043	100	25.0	72	100.0
	A2	.036	.043	100	25.0	432	100.0
	AA1	0.082	.100	400	8.2	0.05	4.1
	AA2	.156	.185	400	8.2	0.07	7.4
Et_3N+EtI	B1	0.036	.041	100	60	576	100.3
	B2	.036	.041	100	60	576	99.9
	BA1	0.165	.173	100	6.0	5.5	2.3
	BA2	.168	.173	100	6.0	12	4.7
	BB1	0.576	.645	150	25.0	0.65	2.1
	BB2	.235	.255	60	25.0	1.6	4.1
	BC1	0.121	.149	75	56.8	0.35	5.4
	BC2	0.122	.151	75	56.8	0.42	6.6
	BD1	0.079	.110	60	78.2	0.12	6.4
	BD2	.032	.044	25	78.2	0.17	8.6
Et_3N+iPrI	C1	0.369	.375	37	61	11.8	2.4
Me_3N+EtBr	D1	0.223	.302	300	25.0	504	100.0
	D2	.223	.302	300	25.0	624	100.0
	DA1	0.223	.302	100	5.8	0.75	5.2
	DA2	.223	.302	100	5.8	1.0	6.3
	DB1	0.223	0.302	100	25.2	0.2	4.4
	DB2	.223	.302	100	25.2	0.15	5.7

*Based on the amine reactant

Purification And Characterization Of Quaternary Ammonium Salts

Each quaternary ammonium salt was purified by recrystallization. The solvent systems from which the quaternary ammonium salts were recrystallized are listed in TABLE VII.

TABLE VII

PURIFICATION OF QUATERNARY AMMONIUM SALTS

Quaternary Ammonium Salt	Solvent
Methyltriethylammonium iodide	Isopropanol
Tetraethylammonium iodide	Ethanol
Isopropyltriethylammonium iodide	Ethyl acetate - Isopropanol
Ethyltrimethylammonium iodide	Ethyl acetate-Isopropanol

With the exception of isopropyltriethylammonium iodide the crude salts were white and the purified quaternary salts were white well-defined crystals. The purified salts were stored in the dark or in amber bottles as it was found that sunlight caused decomposition. Tetraethylammonium iodide and methyltriethylammonium iodide were not hygroscopic, but ethyltrimethylammonium bromide took up water readily' In marked contrast to the others, the crude isopropyltriethylammonium iodide was brownish-black in color. Recrystallization failed to remove all of the color and the recrystallized salt consisted of a light brown powder instead of the well defined crystals obtained for the other quaternary salts studied. All salts were dried under vacuum at 56°C in an Aberhalden drying pistol over phosphorous pentoxide immediately before use.

Even the most stable quaternary ammonium salts tend to decompose if they are not handled carefully. A yellow discoloration of the recrystallization solvent or the purified salt indicates decomposition. While the solutions and purified samples of isopropyltriethylammonium iodide were persistently discolored, only rarely was there even slight discoloration of the other quaternary halides. Any sample which discolored during purification was discarded.

Since quaternary ammonium salts decompose on heating, melting points cannot be used as a criterion of purity. Determination of the anion provides a more reliable criterion of the purity. The anion may conveniently be determined by either of the following methods. The halide may be converted to the corresponding hydroxide and titrated with standard acid or the halide may be determined directly by titration with standard silver nitrate solution using eosin indicator. A third method of estimating purity is provided by a comparison of the theoretical and experimental recoveries of ammonia from Kjeldahl digestions of the quaternary salts. All of the above analytical methods have been used to establish the purity of the quaternary ammonium salts which in all cases was satisfactory with the exception of isopropyltriethylammonium iodide for which consistently low values were obtained. The analytical results, expressed as per cent anion found with standard deviations, are tabulated in TABLE VIII.

TABLE VIII

DETERMINATION OF QUATERNARY AMMONIUM ANION AND RECOVERY OF AMMONIA FROM KJELDAHL DIGESTIONS

Method	Quaternary Salt	Per Cent Anion Found	No. of Analyses
Titration of Hydroxide	$(Me\ Et_3N)^+OH^-$	99.4	1
	$(Et_4N)^+OH^-$	99.0 ± 0.2	20
	$(iPr\ (Et)_3N)^+OH^-$	97.0 ± 0.3	2
	$(EtMe_3N)^+OH^-$	99.9 ± 0.4	2
Titration of Iodide	$(Et_4N)^+I^-$	98.7 ± 0.1	4
Recovery of Ammonia from Kjeldahl digestions	$(MeEt_3N)^+I^-$	97.5 ± 0.9	12
	$(Et_4N)^+I^-$	96.6 ± 1.8	30
	$(iPrEt_3N)^+I^-$	94.7 ± 1.0	3
	$(EtMe_3N)^+I^-$	97.5 ± 1.2	25

Solubilities

Ideally the solubilities of the quaternary ammonium salts should be determined in the reaction mixtures themselves. Since this was impossible, the solubilities of all quaternary salts prepared were determined in the individual components of the reaction system, i.e., amine, halide and benzene. After equilibration of a given quaternary ammonium salt in a specific component of the reaction mixture for several days under anhydrous conditions, five ml. aliquots of the solvent were transferred to a weighed container. Subsequently the solvent was removed, the container was dried over phosphorous pentoxide and reweighed. No attempt was made to measure the solubility of ethyltrimethylammonium bromide in trimethylamine. In all cases the solubilities of the quaternary ammonium halides in the constituents of the reaction mixture were found to be of the same order of magnitude as the weighing errors, i.e., zero.

Examination of Bond Formation Reactions for Partial Elimination or Displacement

<u>The System Triethylamine - Ethyl Iodide</u>:- Tetraethylammonium iodide (2.0 gm.) was equilibrated in a solution of 10.0 gm. trimethylamine and 100 ml. of thiophene-free benzene. This mixture was stirred at $25^{\circ}C$ for nine hours, an interval

seven times longer than that required for four per cent reaction at the same temperature. The recovered quaternary salt was recrystallized from ethanol and dried. Four 250 mg. samples were digested by the usual Kjeldahl procedure.

The results of these analyses were: calculated for pure tetraethylammonium iodide: N, 5.45 per cent; found 5.24, 5.31, 5.28, 5.19 per cent. These values are in good agreement with the average value, 5.26 per cent, obtained for 31 digestions of tetraethylammonium iodide from which nitrogen samples were prepared. The experimental values may also be compared with 6.52 per cent calculated for ethyltrimethylammonium iodide, the product of complete exchange. The close agreement between the experimental results and the per cent nitrogen calculated for the starting material and the values observed in routine digestions strongly indicate that little, if any, displacement or elimination occurred during the equilibration period.

The System Trimethylamine - Ethyl Bromide:- Ethyltrimethylammonium bromide (2.4 gm.) was equilibrated with 26.0 gm. triethylamine in 100 ml. of thiophene-free benzene at 30°C. After stirring the mixture for two hours, the quaternary bromide was recovered, recrystallized from an ethyl acetate - isopropanol mixed solvent and dried. Six samples were degraded to ammonia by the usual Kjeldahl procedure.

The analytical results were: calculated for pure ethyltrimethylammonium bromide, 8.34 per cent N ; found 8.07, 8.22, 8.05, 8.12, 8.11 and 8.16 per cent N. These recoveries are very close to the average value, 8.15 per cent, found for 25 digestions of the pure quaternary bromide. The experimental recoveries would have been much lower if appreciable exchange had occurred during the equilibration period, i.e., 6.76 per cent if the material recovered had been tetraethylammonium bromide, a possible exchange product. These results demonstrate that the reaction of trimethylamine with ethyl bromide is a unidirectional process.

Kjeldahl Digestion Procedure

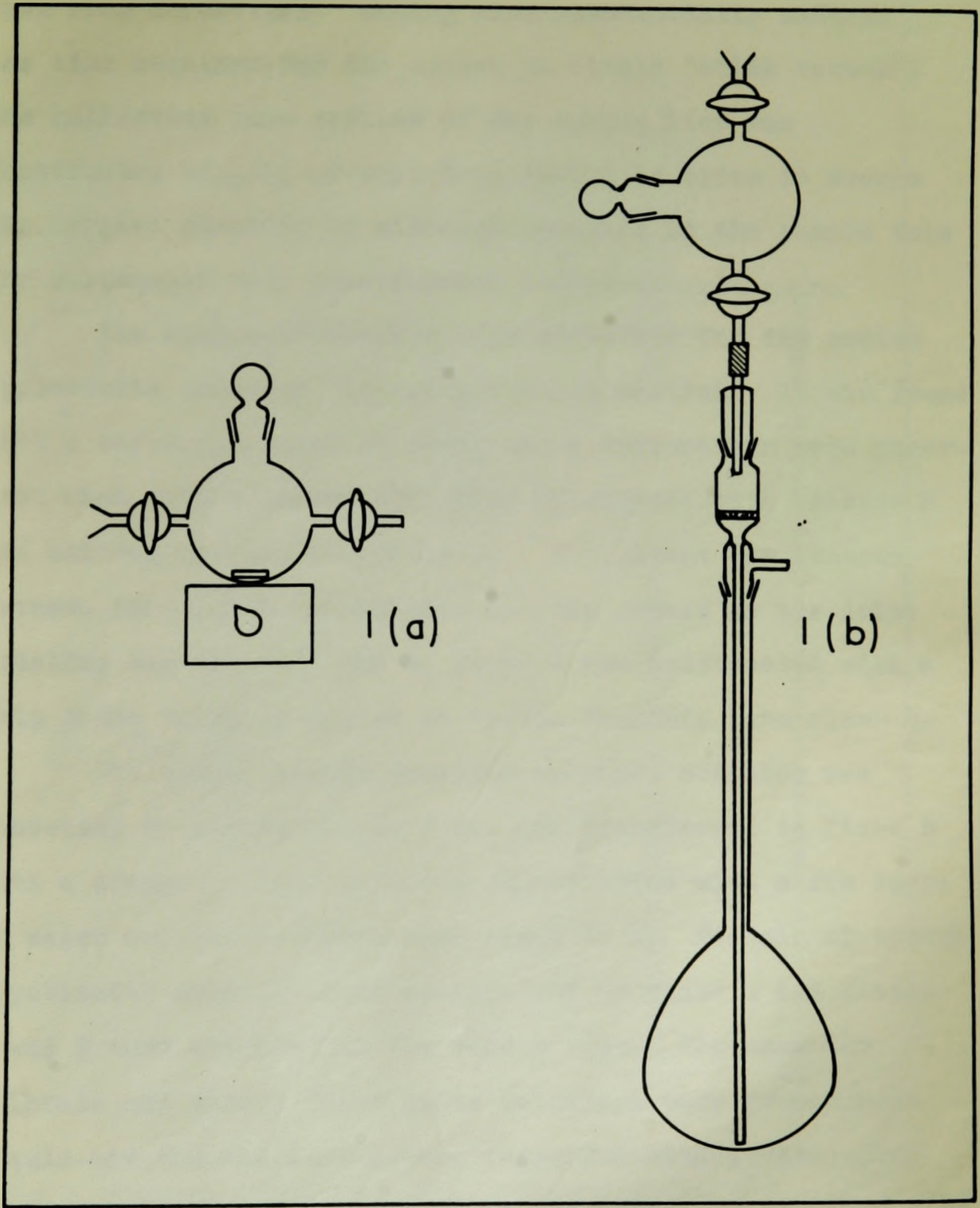
Approximately one millimole of the quaternary ammonium salt and 500 mg. of silver hydroxide were transferred to the reaction flask shown in Fig.1 (a). Distilled water (20 ml.) was added, the flask was stoppered and the mixture was stirred for one hour using a magnetic stirrer. The reaction flask was then attached to the filtering system, Fig.1 (b), by means of a short length of rubber tubing. Suction was applied to the system and the solution of quaternary hydroxide was filtered through a sintered-glass filter, into a Kjeldahl flask containing 15 ml. of 0.1N sulphuric acid. The reaction flask and filter were washed four times with five ml. water and the washings were combined with the filtrate. The use of this closed system for reaction and filtration eliminated mechanical losses and prevented losses due to decomposition of the quaternary hydroxide. It was found that approximately 0.2 per cent of the quaternary ammonium hydroxide decomposed during the reaction of the halide with silver hydroxide and the subsequent filtration. The water was then removed from the quaternary ammonium sulphate by vacuum distillation using a water pump. To the dry sulphate 0.350 gm. mercuric oxide, 8.50 gm. potassium sulphate, 14 ml. sulphuric acid and an "antibumper" were added. After fitting the flask with a Kjeldahl trap, this mixture was boiled vigorously for twelve hours over a microburner.

At the end of the digestion period the mixture was cooled to room temperature, diluted with 100 ml. of water and neutralized with 60 ml. of a 30 per cent solution of sodium hydroxide containing five per cent sodium thiosulphate. The ammonia was distilled into 20 ml. of 0.1 N sulphuric acid. A stream of purified hydrogen was bubbled through the boiling solution to insure rapid removal of ammonia. The sulphuric acid solution was boiled to expel carbon dioxide and back titrated with standard sodium hydroxide using methyl red as indicator. The solution was then made slightly acid to prevent loss of ammonia.

The average recoveries of ammonia from the Kjeldahl digestions were: methyltriethylammonium iodide, 97.5 ± 0.9 per cent, tetraethylammonium iodide, 96.6 ± 1.8 per cent ethyltrimethylammonium bromide, 97.5 ± 1.2 per cent and isopropyltriethylammonium iodide, 94.7 ± 1.0 per cent. The experimental error is expressed in terms of the standard deviation.

Preparation of Nitrogen Samples

The ammonia samples were oxidized to nitrogen in the vacuum line shown in Fig.2. High speed mechanical and mercury diffusion pumps were used to obtain pressures of 10^{-6} mm Hg, "stick vacuum", which were measured with a McLeod gauge. The line was operated continuously when samples were being prepared. A water pump was used to evacuate the reaction flasks and sample tube section of the line to approximately 10 mm. Hg before they were turned into the main line. The number of stopcocks was reduced to a minimum to permit baking of the vacuum line with a torch during the preparation of nitrogen samples. This was found to be the most effective method for keeping the vacuum line clean and hence the sample



1 (a)

1 (b)

FIGURE 1. APPARATUS FOR PREPARATION OF KJELDAHL SAMPLES

free from impurities. Baking also substantially reduced the time required for the system to attain "stick vacuum". The collection tube section of the sample line was constructed largely of capillary tubing in order to secure the largest quantity of nitrogen possible in the sample tube for subsequent mass spectrometer analyses.

The design of flask A, the container for the sodium hypobromite solution, deserves special mention. It was found that a carbon-chlorine compound and a hydrocarbon were generated when sodium hypobromite came in contact with Apiezon N and silicon grease, respectively. To prevent any contact between the sodium hypobromite and the grease on the joint coupling the flasks A and B, flask A was constructed with a drip joint which projected as far as possible into flask B.

The slightly acid ammonium sulphate solution was condensed to approximately 3 ml. and transferred to flask B with a dropper. The flask was rinsed twice with a few drops of water and the washings were added to B. Ten ml. of sodium hypobromite solution were transferred to flask A and flasks A and B were attached to the vacuum line. The ammonium sulphate and sodium hypobromite solutions were frozen with liquid air and the flasks were evacuated with a water pump C. Then stopcock d was closed, c was opened to the main line

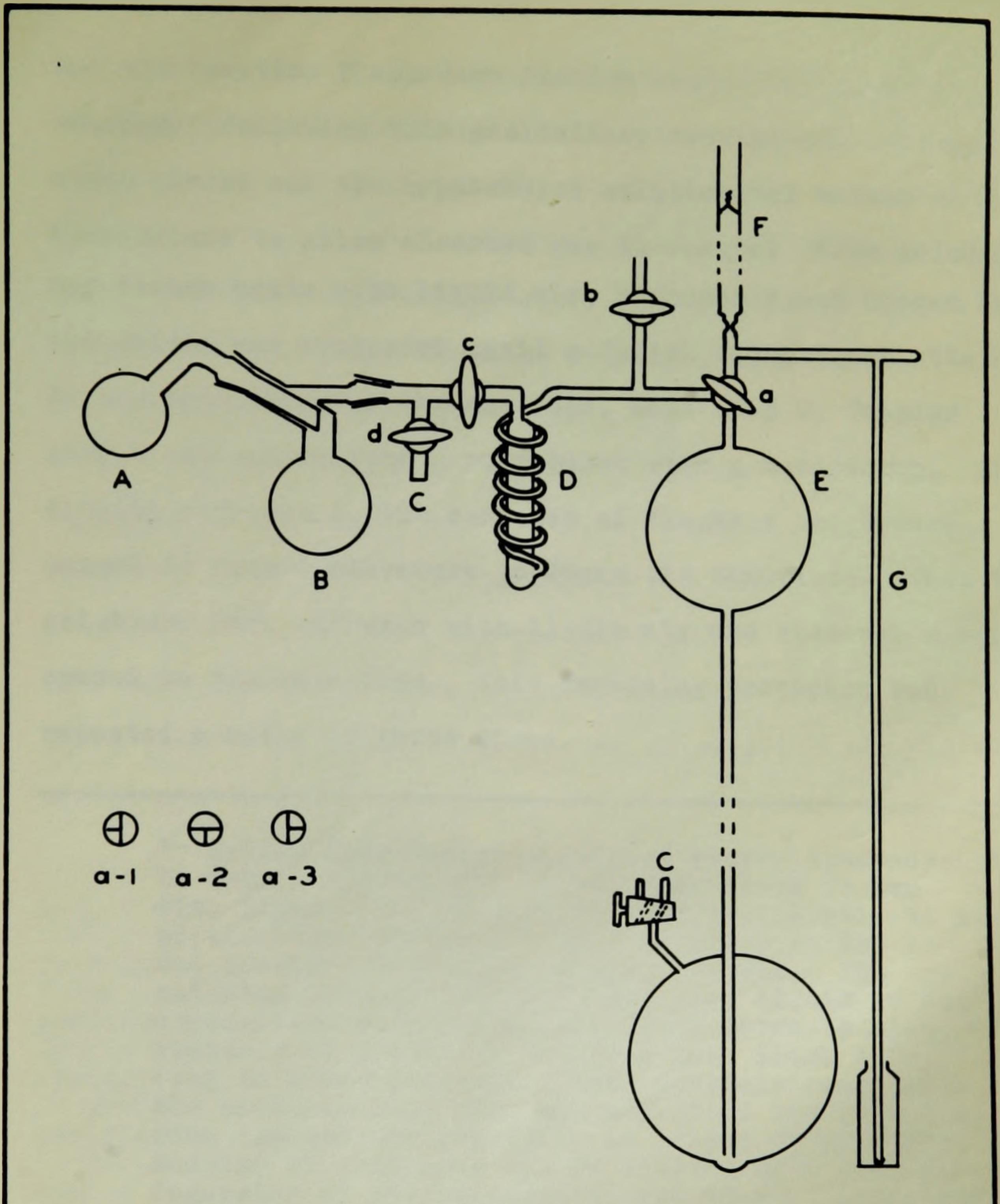


FIGURE 2. VACUUM APPARATUS FOR PREPARATION OF NITROGEN SAMPLES

and the reaction flasks were further evacuated for several minutes. Following this preliminary evacuation, stopcock c was closed and the hypobromite solution was warmed to room temperature to allow absorbed gas to escape*. The solution was frozen again with liquid air, stopcock c was opened and the system was evacuated until a "stick vacuum" was attained. During this interval the manifold, coil trap D, Toepler pump E and sample tube F were baked with a hand torch. After closing stopcock c, the contents of flasks A and B were warmed to room temperature to degas the solutions. Then the solutions were refrozen with liquid air and stopcock c was opened to the main line. This degassing procedure was repeated a total of three times.

---

* Sodium hypobromite solutions have a tendency to form a supercooled liquid when first frozen with liquid air and then to change violently to a crystalline structure. This tendency is due to the presence of oxygen in the solution. The solution changes from a supercooled liquid to the crystalline state with sufficient force to blow crystals of sodium hypobromite into flask B or even to break flask A. These crystals react with the ammonium salt when the solutions are warmed to room temperature resulting in a loss of nitrogen. Bumping of this sort can be avoided by a preliminary degassing of the hypobromite solution as described in the text.

After the final degassing, stopcock c was closed and the sodium hypobromite was transferred to flask B by inverting flask A. The reaction mixture in flask B was warmed with a beaker of water at 40 - 50°C to hasten the reaction, and then was frozen with liquid air. Ten minutes at this temperature served to condense any impurities before collection of the nitrogen sample.

A liquid air Dewar was placed on the coil trap. Stopcock b was closed, the three-way stopcock was rotated from position a-1 to a-2 and stopcock c was opened. The nitrogen was drawn from the reaction flasks into the Toepler pump and purified by forcing it through the spiral trap three times. Stopcock a was turned to position a-3 and the purified nitrogen was compressed into the sample tube.

After the third compression, stopcock a was rotated to a-2, c was opened and a second portion of nitrogen was drawn from the reaction flasks into the Toepler pump. It was purified and compressed into the sample tube as described above. While the second portion of nitrogen was being purified and collected, the reaction mixture in flask B was warmed to room temperature to permit nitrogen trapped in the frozen liquid to escape. After refreezing the mixture for ten minutes, this final quantity of nitrogen was purified and transferred to the sample tube as described previously. Approximately 95 per cent of the nitrogen evolved was collected in the sample tube by this procedure. After the last nitrogen was compressed into the sample tube, the gas pressure was read from the manometer G and the tube was sealed off with a torch.

The Sodium Hypobromite Oxidation of Ammonia

The oxidation of the ammonium ion by sodium hypobromite was studied quantitatively. The sample line and tube were calibrated and the procedure was standardized by oxidizing 41.66 mg. samples of ammonium chloride with ten ml. aliquots of sodium hypobromite. After the solutions had been degassed, they were mixed and the nitrogen generated was quantitatively transferred to the sample tube as described above. The pressure and temperature of the gas in the sample tube were noted. The extent of the oxidation reaction was computed from the observed and calculated gas pressures at the reaction temperature. The ammoniacal materials recovered from the Kjeldahl digestions of tetraethylammonium iodide and triethylamine were oxidized in a similar manner. The results expressed as per cent oxidation to nitrogen are given in TABLE IX.

TABLE IX

OXIDATION OF AMMONIA TO NITROGEN

Starting Material	Per Cent Oxidation
NH_4Cl	98.2
	98.8
	94.8
	98.3
Et_4NI	100.6
	102.5
	100.3
	97.6
Et_3N	95.7
	97.4
	100.9
	101.1

These results show that ammonia is quantitatively oxidized to nitrogen by sodium hypobromite.

Mass Spectrometry

All nitrogen samples were analyzed in the 180° direction focusing mass spectrometer described by Thode, Graham and Ziegler(58). The ion currents were amplified by a total feedback d.-c. amplifier using a 954 electrometer in the first stage of amplification. The output was fed into a Leeds and Northrup Speedomax recorder. Automatic magnetic scanning was used throughout.

Sample Analysis

Nitrogen isotope effects were computed from the 28/29 ratios of the nitrogen prepared from completely reacted and partially reacted quaternary ammonium salts. These effects were obtained indirectly by measuring the isotopic ratio of each sample relative to purified tank nitrogen and directly by intercomparing the samples themselves. An individual determination of either tank or sample nitrogen was composed of eight spectrograms. A spectrogram was obtained by first scanning over the 28 and 29 mass peaks and then, by reversing the scan, over the 29 and 28 peaks. The 28 and 29 mass peaks in each spectrogram were averaged before the 28/29 ratio was calculated. This served to compensate for short term fluctuations in the spectrometer.

In the indirect determinations the isotopic ratios of the samples were measured relative to tank nitrogen to compensate for the day-to-day variations in the electronic characteristics of the spectrometer. A sample analysis was composed of the sequence, tank-sample-tank-sample-tank, etc. performed as rapidly as possible. A minimum sample analysis consisted of the series, tank-sample-tank.

In the direct determinations the isotopic ratios of the samples were measured relative to each other, i.e., the nitrogen from a partial reaction against that from the complete reaction or samples prepared from partial reactions against each other. A typical intercomparison of this type could consist of the sample sequence, complete-partial-complete-partial, etc.

A sample analysis was considered significant only if the data successfully met prescribed statistical tests based on analysis of variance methods. From the data accepted, 95 per cent confidence intervals were computed as the difference between the means calculated for the tank and sample nitrogen in the indirect method, or in the direct method as the difference between the means of the two samples being intercompared. The means were computed as the average values of all observations in a given population, i.e., sample or tank nitrogen. The isotope effects were calculated from these confidence intervals.

Each sample was examined for impurities over the mass range 28 to 45. Very small peaks corresponding to masses 30, 32, 39, 40 and 44 were observed in all nitrogen samples. With the exception of the peak at mass 32, due to oxygen, these were 0.5 to 2.0 cm in height on the most sensitive shunt of the electrometer. The percentage composition of a typical nitrogen sample is given in TABLE X.

TABLE X

THE PERCENTAGE COMPOSITION OF A TYPICAL NITROGEN SAMPLE

Mass Peak	Height cm.	Electrometer Sensitivity	Percentage in Sample
28	23.2	5.0	99.14
29	17.0	0.05	0.73
30	1.5	0.01	0.01
32	8.8	0.01	0.08
39	3.0	0.01	0.03
40	1.6	0.01	0.01
44	1.5	0.01	0.01

Previous workers, who have studied nitrogen of natural abundance, have observed that the 30 mass peak is invariably larger than expected for $N^{15} - N^{15}$(62). Since this peak is small and difficult to measure, it is generally disregarded and the abundance of N^{15}, if desired, is calculated from the 28 and 29 peaks. This abnormal peak at mass 30 has been shown to be due to nitric oxide. Riley _et al_ have found that nitrous oxide, mass 44, is formed in the oxidation of ammonium salts, hydrazine and hydroxylamine with sodium hypobromite(63). Under electron impact in the spectrometer nitrous oxide dissociates to nitric oxide. It has been observed in this laboratory that nitric oxide can be generated in the spectrometer if oxygen is present.

The concentration of the oxygen peak, mass 32, varied from 0.03 to 0.15 per cent. A portion of this oxygen, no doubt, was evolved by the sodium hypobromite solution, which is known to slowly evolve oxygen(61). Most of the oxygen, however, was introduced mechanically when a new sample tube was placed on the spectrometer since it was noted that oxygen is pumped out of the spectrometer quite slowly. In the time allowed to evacuate the free space above the break seal of the sample tube, effectively all of the nitrogen was pumped out, but a significant quantity of oxygen remained, probably absorbed on the glass. This oxygen was then carried into the spectrometer with the nitrogen sample. The fact that much less oxygen was found in a sample during subsequent analyses bears out this conclusion.

The mass peaks observed at 39, 40 and 44 were due to doubly charged potassium, argon, and carbon dioxide or nitrous oxide, respectively.

There was no evidence in any of the samples of hydrocarbons or amines which would result from incomplete Kjeldahl digestions.

Reproducibility of Sample Analyses

The reproducibility of the sample analyses, i.e., the 28/29 isotopic ratios, can be influenced both by slight variations in the degradation procedure used to convert the quaternary ammonium salts to nitrogen, and by variations in the electronic characteristics of the mass spectrometer. The precision of the spectrometer for a single analysis was 0.1 per cent.

The reproducibility attained in this investigation is illustrated in TABLE XI and XII in which the analytical data are recorded as the 28/29 isotopic ratios normalized to a value of 136.20 for tank nitrogen. The results of four individual analyses of the same sample over a two month period are tabulated in TABLE XI.

TABLE XI

NORMALIZED 28/29 RATIO OF A SAMPLE ANALYZED AT DIFFERENT TIMES

Sample	Normalized 28/29 Ratio
B2-2	135.29
	135.23
	135.35
	135.21
	135.27 ± 0.06

The normalized 28/29 ratios of the nitrogen from the complete reactions, triethylamine with ethyl iodide and triethylamine with methyl iodide, and from the partial reaction of triethylamine with ethyl iodide at 78°C are recorded in TABLE XII. Since amine from the same stock was used in the complete reactions, triethylamine-ethyl iodide and triethylamine-methyl iodide, the nitrogen samples prepared from these salts should have the same isotopic ratios. The errors are expressed as standard deviations relative to the value of the isotopic ratios.

Beliskov <u>et al</u>(62) have carefully measured nitrogen isotopic abundances in a simultaneous collection mass spectrometer. These authors reported an error on a single observation of ±0.04 per cent for repeated measurements of the abundances of a single sample, an error on a single observation of ± 0.18 per cent in the measurement of the abundances of different nitrogen samples prepared from the same specimen of slightly enriched ammonium nitrate. Therefore, according to these workers, it would appear that the chemistry involved is the limiting factor rather than the mass spectrometry. In this study the precision of the spectrometer measurements was better than that reported by Beliskov even though the direction focusing mass spectrometer used lacked the precision attainable with a simultaneous collection instrument. This high precision was attained by exercising extreme care in the preparation and mass spectrometer analyses of the nitrogen samples. Despite this high precision, however, it will be apparent from the Results that more definite conclusions, particularly with respect to the influence of temperature on the magnitude of the isotope effect, would have been possible if the isotopic abundances could have been measured with greater precision.

TABLE XII

NORMALIZED 28/29 RATIOS OF NITROGEN FROM COMPLETE AND PARTIAL REACTIONS

System	Sample No.	Normalized 28/29 Ratios	
		Single Analysis	Ave. Value
Et_3N-MeI (Complete Reaction)	A1-1	135.36	135.36
	-2	135.38	
		135.11	135.25
	A2-1	135.28	135.28
	-2	135.28	135.28
	-3	135.42	135.42
			135.32 ± 0.06
Et_3N-EtI (Complete Reaction)	B1-1	135.31	135.31
	-2	135.32	135.32
	B2-1	135.45	135.45
	-2	135.29	
		135.23	
		135.35	
		135.21	135.27
	-3	135.39	135.39
			135.35 ± 0.06
Et_3N-EtI (Partial Reaction, 78°C)	BD1-1	135.64	135.64
	-2	135.54	135.54
	-3	135.52	
		135.55	135.55
	BD2-1	135.71	135.71
	-2	135.60	135.60
			135.61 ± 0.06

CALCULATIONS

Introduction

Analysis of variance techniques were used to evaluate the mass spectrometer data. These techniques are particularly useful in the assessment of data from small numbers of samples. The total variation observed in experimental data is divided into components associated with possible sources of variability whose relative significance can be evaluated. These statistical methods are described in detail by Bennett and Franklin (64).

The variance is defined as the sum of squares of the deviations from the mean divided by one less than the number of observations, i.e.,

$$s^2 = \sum_{i}^{n} \frac{(x_i - \bar{x})^2}{n - 1} \qquad (59)$$

where s^2 is the sample variance, x_i is a value of the random variable, $\bar{x}$ is the mean of a set of observations, and the quantity $(n - 1)$ is called the degrees of freedom. The quantity s^2 is the best estimate of the true variance of the population, σ^2, and $\bar{x}$ is used in place of μ, the true mean of the parent population. The square root of the variance, s, is called the standard deviation.

The factor n-1 is introduced to compensate, on the average, for the fact that the sum of squares of the deviations has a minimum value when $\bar{x}$ is used instead of μ, the true mean. The degree to which $\bar{x}$ reflects the average depends directly on the number of observations used in computing it. Similarly, the degree to which s^2 reflects the variance, σ^2, depends on the number of results on which it is based. Therefore, the estimate of s^2 is computed from n-1 rather than n observations and it is said to have n-1 degrees of freedom.

If the samples, i.e., classes, are drawn from a homogeneous population, the average values for each sample will not be identical since the sample averages will reflect the variation of the parent population. The variation between the samples, however, should be consistent with the population variance as indicated by the variation within the individual observations. If the "between sample variation" is significantly greater than the "within sample variation", it indicates that the samples have been drawn from populations with different average values.

A variance is computed for each of these components of variation by dividing the appropriate sums of squares by the corresponding degrees of freedom. Since these estimates are independent of each other they need not be identical in value. The Null Hypothesis is tested to determine whether the difference between the "within sample variance" and the "between sample variance" is significant. Since the Null Hypothesis assumes that the samples are all drawn from a common population, the variances associated with each component of variation should not differ more than expected for the corresponding number of degrees of freedom. This hypothesis is tested by calculating the Variance Ratio, F,

$$F = \frac{s_1^2}{s_2^2} , \qquad (60)$$

where s_1^2 and s_2^2 are the "between class variance" and the "within class variance", respectively.

If the value of F is greater than expected on the basis of the Null Hypothesis, it is concluded that there is a significant difference between the samples and that they represent different populations.

Analysis of Variance

The computation of the Variance Ratio from the mass spectrometer data may be developed as follows. The total number of observations, N, is equal to the product of the number of replicate results, n, and the number of classes, p.

The observations are denoted by $x_{i\gamma}$ where $\gamma = 1, \ldots, n$ and $i = 1, \ldots, p$. The analytical data being tested has the form,

A_1	B_1	A_2	B_2	A_3	$\cdots$
x_1	x_1	x_1	x_1	x_1	
x_2	x_2	x_2	x_2	x_2	
$\vdots$	$\vdots$	$\vdots$	$\vdots$	$\vdots$	
x_n	x_n	x_n	x_n	x_n	

where the classes A and B denote successive observations of the isotopic ratios of tank and sample nitrogen or of the intercomparisons between different samples. In most analyses eight spectrograms were measured, hence, $n = 8$. The minimum value of p in any analysis was three, that is, a minimum analysis consisted of the classes, tank - sample - tank.

It is assumed that the average value of each observation in a particular class is the sum of an overall mean, μ, and a variation from that mean, ϵ_i, i.e.,

$$\text{ave.}\,(x_{i\gamma}) = \mu + \epsilon_i\,, \qquad (61)$$

and that all observations are normally distributed about these average values with a common variance, σ^2. Each class is composed of n observations from a normal distribution with mean, $\mu + \varepsilon_i$, and variance, σ^2.

The means of the n observations of the i^{th} class and the N total observations are designated by $\bar{x}_i$ and $\bar{x}$, respectively.

The total variation, S, is given by

$$S = \sum_{i\gamma}(x_{i\gamma} - \bar{x})^2 \qquad (62)$$

$$= \sum_{i\gamma} x_{i\gamma}^2 - \frac{T^2}{N} \qquad (63)$$

where T is the sum of all N = np observations, i.e., $T = \sum_{i\gamma} x_{i\gamma}$. The overall sum of squares S is associated with N-1 degrees of freedom.

The variation between classes, S_1, is given by the formula

$$S_i = n \sum_i (x_i - \bar{x})^2 \qquad (64)$$

$$= \sum_i \frac{T_i^2}{n} - \frac{T^2}{N} \qquad (65)$$

where $T_i = \sum_\gamma x_{i\gamma}$, the sum of the n observations in the i^{th} class. Since S_i is computed from the deviations of p independent class means from the overall mean, it has p-1 degrees of freedom.

$S_{\gamma(i)}$, the sum of squares within classes, is computed from the deviations of the $N = np$ observations from the p class means, hence, has $N - p$ degrees of freedom. $S_{\gamma(i)}$ is given by

$$S_{\gamma(i)} = \sum_{i\gamma} (x_{i\gamma} - \bar{x}_i)^2 \qquad (66)$$

$$= \sum_{i\gamma} x_{i\gamma}^2 - \sum_i \frac{T_i^2}{n} \qquad (67)$$

$$= S - S_i \qquad (68)$$

These statements can be summarized in an analysis of variance table, Table XIII.

Table XIII

ANALYSIS OF VARIANCE TABLE

Source of Estimate	Sum of Squares	Degrees of Freedom	Mean Square	Ave. Mean Square
Between Classes	S_i	$p - 1$	$S_i/(p-1)$	$\sigma^2 + n\sigma_\varepsilon^2$
Within Classes	$S_{\gamma(i)}$	$N - p$	$S_{\gamma(i)}/(N-p)$	σ^2
Total	S	$N - 1$		

$S_{\gamma(i)}/(N-p)$ is a joint estimate of σ^2, the variation within the p classes, and $S_i/(p-1)$ is an estimate of $\sigma^2 + n\sigma_\varepsilon^2$, where σ_ε^2 is the variance of the population of effects from which the ε_i were drawn. The estimate of the basic variance σ^2 is called the error variance estimate. Under the Null Hypothesis $\sigma_\varepsilon^2 = 0$ and both estimates have the same value σ^2. This hypothesis is tested by computing the ratio of the between classes estimate to the within classes estimate. Therefore, the Variance Ratio is given by

$$F = \frac{S}{p-1} \Big/ \frac{S_{\gamma(i)}}{N-p} \qquad (69)$$

This value is compared with that computed for the percentage points of the F distribution, $F_{(p-1),\,(N-p),\,\alpha}$, where α is the significance level. All analytical data were tested at the five percent level of significance, that is, for $\alpha = 0.05$. If the calculated value of F is greater than that expected it is concluded that the classes, i.e., tank and sample nitrogen, were drawn from different populations. At this level of significance the conclusion that the samples are different would be expected to be wrong five percent of the time. Any analysis in which the calculated value of F was less than $F_{(p-1),\,(N-p),\,0.05}$ was rejected.

Since the number of observations in any analysis was relatively small, the results were further examined statistically before confidence intervals were computed.

This procedure is necessary since the chances that the true mean falls outside the calculated confidence interval are not independent. This situation arises because a common value of the standard deviation is used in the evaluation of the confidence interval. To test for this event the ranges of the observed classes are compared and classified. Basic to this treatment is the assumption that all sample means are normally distributed and have an average value μ from a population with a normal variance, σ^2/n. The sample variance, s^2/n, must be used as an estiamte of the true variance, σ^2/n, s^2 being obtained as the within sample variation.

Comparison of Means

Again it is assumed that the means of all classes are equal, the estimate of the mean being given by $\bar{x}_i = T_i/n$. This hypothesis can be tested by determining the standard deviation of the means using the studentized range. The greatest comparison difference is given by

$$\sqrt{s^2/n} \cdot W_{(N-p),\alpha} \tag{70}$$

where $W_{(N-p),\alpha}$ is the value of the studentized range for N-p degrees of freedom and $\alpha = 0.05$.

The class means, $\bar{x}_i$, are arranged in order of increasing magnitude and the value calculated for

$\sqrt{s^2/n} \cdot W_{(n-p),\alpha}$ is compared with the greatest differences between the means of the subgroups. If the greatest differences of the means within a subgroup, i.e., tank or sample nitrogen, are less than $\sqrt{s^2/n} \cdot W_{(n-p),\alpha}$ and if the least difference between the means of the two subgroups is greater than this value, it is concluded that the subgroups arose from different populations and are significantly different at the five percent level.

If these conditions are not met, no action is taken until the means have been classified by the ranking procedure developed by Tukey. This procedure is more sensitive than the comparison of means and, consequently, is a better test of the stability of the spectrometer during the analysis.

Classification of Means

The means are first ranked by a t-test at a significance level of five percent, i.e., $\alpha = 0.05$. The difference that must not be exceeded by adjacent means within a subgroup is given by

$$t_{(N-p),\alpha} \cdot s_{\bar{x}_i} \cdot \sqrt{2}, \qquad (71)$$

where $t_{(N-p),\alpha}$ is the value of the percentage points of the t-distribution for N-p degrees of freedom for $\alpha = 0.05$, and

$$s_{\bar{x}_i} = \sqrt{\frac{s^2}{n}} \qquad (72)$$

This value is then compared with the difference between successive means arranged in increasing order of magnitude. Any gap between two adjacent means greater than the calculated difference is considered to be a division between two groups. If the class means have been drawn from different populations, they divide into two subgroups, i.e., tank and sample means; that is, the difference between successive class means in either subgroup are less than the value $t_{(N-p),\alpha} \cdot s_{\bar{x}_i}\sqrt{2}$ while the least difference between the subgroups is greater than this value. The classes of a subgroup in which the differences between successive means are less than $t_{(N-p),\alpha} \cdot s_{\bar{x}_i}\sqrt{2}$ are considered to represent a single population at the significance level chosen.

If a subgroup contains a maximum of two means the process terminates and the groups are declared to be significantly different.

For subgroups containing three or more class means the grand mean, $\bar{x}_m$, of the group is computed. Then, the largest difference, d_L, between the class means $\bar{x}_i$ and the grand mean is determined, i.e.

$$d_L = \bar{x}_i - \bar{x}_m \qquad (73)$$

When a subgroup contains exactly three classes, t is computed as

$$t = \left(\frac{d_L}{s_{\bar{x}_i}} - \frac{1}{2}\right) \Big/ 3\left(\frac{1}{4} + \frac{1}{m}\right) \qquad (74)$$

where m is the number of degrees of freedom in the estimate of the error variance, i.e., N-p. If the subgroup contains more than three classes, equation (74) takes the form

$$t = \left(\frac{d_L}{s_{\bar{x}_i}} - \frac{6}{5}\log_{10} k\right) \Big/ 3\left(\frac{1}{4} + \frac{1}{m}\right) \qquad (75)$$

where k is the number of means in the subgroup.

If the calculated value of t is less than the critical value t_α, the value of the standardized normal variable for $\alpha = 0.05$, it is concluded that the means in the subgroup arise from the same population. On the other hand, if the calculated value of t exceed t_α, it indicates that the means come from different populations and that the means should be divided into further groups. The critical value of t at $\alpha = 0.05$ is 1.96.

Finally the homogeneity of each subgroup of three or more class means is tested by a F-test. An estimate of the variance, s_q^2, is obtained by dividing the sum of squares of the deviations of the class means from the grand mean by the corresponding number of degrees of freedom, i.e.,

$$s_q^{\,2} = \frac{\Sigma(\bar{x}_i - \bar{x}_m)^2}{q-1} \qquad (76)$$

where q is the number of class means in the subgroup. This value is compared with the estimate of the variance obtained earlier by a F-test,

$$F = \frac{s_{\bar{x}_i}^{\,2}}{s_q^{\,2}} \qquad (77)$$

The calculated value of F is compared with that for $F_{(N-p),\,(q-1),\,\alpha}$. To obtain a five percent significance test α is set equal to 0.025 since the larger number is placed in the numerator. If the calculated value of F is less than $F_{(N-p),\,(q-1),\,\alpha}$, it is concluded that the subgroups are drawn from two different homogeneous populations.

At this point the data was assessed on the basis of the above tests. If either subgroup failed to pass these tests, those classes which fell outside the prescribed limits were rejected and the calculations were repeated beginning again with the analysis of variance table. In the sample-tank analyses only that analytical data which successfully met the conditions defined by the F-test and the comparison and classification of means were used in the computation of confidence intervals.

Classes of observations were rejected 21 times in 96 mass spectrometer analyses of samples relative to tank nitrogen. Only three analyses were discarded completely. The first and last classes were rejected in ten and three instances, respectively. In the remaining five analyses, two or more classes were discarded.

This statistical procedure was modified somewhat for the intercomparisons between samples. Since the class means of any two such samples were quite close together, the actual magnitudes of the greatest comparison difference and the ranking difference between means of adjacent classes were disregarded. These tests were used, however, to check the scatter of the class means within the respective subgroups. The data from the intercomparisons which passed the F test (equation 69) and the Student t-test (equation 83) were considered significant and used to compute confidence intervals.

Comparison of Subgroup Ratios

Before the confidence intervals were evaluated, the means of the two subgroups, i.e., tank and sample nitrogen, were compared by a Student t-test. For the tank observations the grand total T, and the mean $\bar{X}$, are defined as

$$T_1 = \sum_{i\gamma} x_{i\gamma} \qquad (78)$$

$$\overline{X}_1 = T_1 / N_1 \qquad (79)$$

where N_1 is the number of observations on tank nitrogen. Similarly, for the sample observations the grand total T_2 and mean $\overline{X}_2$ are given by

$$T_2 = \sum_{i\gamma} x_{i\gamma} \qquad (80)$$

$$\overline{X}_2 = T_2 / N_2 \qquad (81)$$

where N_2 is the number of observations on sample nitrogen.

The pooled estimate of the variance s_p^2 is given by

$$s_p^2 = \frac{\sum_{i\gamma} x_{i\gamma}^2 - (T_1^2/N_1) - (T_2^2/N_2)}{N_1 + N_2 - 2} \qquad (82)$$

where $\sum_{i\gamma} x_{i\gamma}^2$ is the total sum of squares, T^2/N is a correction factor and $(N_1 + N_2 - 2)$ defines the associated number of degrees of freedom. The pooled estimate of the standard deviation, s_p, is calculated from equation (82).

A Student t-test is then performed on the assumption that the group variances are equal and that the sample variance can be used in place of the true variance. The quantity t is given by

$$t = \frac{\bar{X}_1 - \bar{X}_2}{s_p \sqrt{(1/N_1) + (1/N_2)}} \qquad (83)$$

The calculated value of t is compared with the expected value $t_{(N_1 + N_2 - 2),\alpha}$ where $\alpha = 0.005$. Since this is a two sided test, only calculated values of t which exceed $t_{(N_1 + N_2 - 2),\alpha}$ show a significant difference between the subgroups at the one per cent level. Data which failed this test were rejected. Failure of the Student t-test was observed only in the data from the intercomparisons between samples, and generally, these analyses also failed the F-test (equation 69).

Computation of Confidence Intervals

A confidence interval is an estimate of an unknown parameter, θ, by two values A and B such that there is a probability $1 - \alpha$ that $A < \theta < B$. A and B are called 100 $(1 - \alpha)$ per cent confidence limits for the parameter θ and the interval between them a 100 $(1 - \alpha)$ per cent confidence interval. There is a 100α per cent 'risk' that the limits A and B do not include the unknown value θ.

A 95 per cent confidence interval is computed from the formula

$$\bar{X}_1 - \bar{X}_2 \pm t_{(N_1 - N_2 - 2),\alpha} \cdot s_p \cdot \sqrt{(1/N_1) + (1/N_2)} \qquad (84)$$

where $\alpha = 0.025$. These confidence intervals are expressed as the differences between the grand means of tank and sample nitrogen or samples which were intercompared.

The analysis of variance techniques used in testing the mass spectrometer data and the calculation of confidence intervals of representative samples are illustrated in Appendix I.

Confidence Intervals for Combined Analyses

In a number of instances a sample was analyzed several times at different periods. In such cases it was convenient to combine this data and express the results as a single confidence interval. Moreover, in the computation of the isotope effects, it was advantageous to combine the data from all samples prepared under the same experimental conditions, ignoring the negligible influence of different reaction times, and express the results as one confidence interval. The procedure for the computation of confidence intervals for combined analyses is briefly outlined in the following sections.

If a total of r analyses are performed, r pairs of means $(\bar{X}_{i_1}, \bar{X}_{i_2})$ are obtained, where $i = 1, \ldots, r$. $\bar{X}_{i_1}$ is the mean of N_{i_1} observations from a normal population with mean μ_1 and variance σ^2. Similarly, $\bar{X}_2$ is the mean of N_{i_2} observations from a normal population with mean μ_2 and variance σ^2. T_{i_1} is the sum of all observations on the first variable in the i^{th} analysis and T_{i_2} is the corresponding sum for the second variable.

$$\text{Let} \quad Z_i = \bar{X}_{i_1} - \bar{X}_{i_2} \qquad (85)$$

a function which has a normal distribution with mean $\mu_1 - \mu_2$ and variance σ_i^2 given by

$$\sigma_i^2 = \sigma^2\left(\frac{1}{N_{i_1}} + \frac{1}{N_{i_2}}\right) \qquad (86)$$

The best estimate of $\mu_1 - \mu_2$ is

$$Y = \sum_{i=1}^{r} \frac{b_i Z_i}{\sum_{i=1}^{r} b_i} \qquad (87)$$

$$\text{where} \qquad b_i = \frac{N_{i_1} \cdot N_{i_2}}{N_{i_1} + N_{i_2}}$$

The mean of Y is $\mu_1 - \mu_2$ while its variance is

$$\sigma_y^2 = \sigma^2 \Big/ \sum_{i=1}^{r} b_i \qquad (89)$$

The pooled estimate of the variance, σ^2, is given by

$$s_p^{\,2} = \frac{\sum_{i\gamma} x_{i\gamma}^2 - \sum_{i=1}^{r} T_{i_1}{}^2/N_{i_1} - \sum_{i=1}^{r} T_{i_2}{}^2/N_{i_2}}{k} \qquad (90)$$

where $\sum_{i\gamma} x_{i\gamma}^2$ is the sum of squares of all observations and

$$k = \sum_{i=1}^{r} (N_{i_1} + N_{i_2}) - 2r \qquad (91)$$

A 95 per cent confidence interval for $\mu_1 - \mu_2$ is given by

$$Y \pm t_{k,\,0.025} \cdot s_p \cdot \frac{1}{\sqrt{\sum_{i=1}^{r} b}} \qquad (92)$$

As in the case of the individual samples this confidence interval for combined analyses gives the difference between the means of samples which are intercompared.

The derivation of equation (92) and the computation of confidence intervals for combined analyses are given in Appendix II.

Computation of Isotope Effects

The experimental data expressed either as isotopic ratios or confidence intervals must be normalized to some ratio assumed for tank nitrogen before isotope effects can be computed. This procedure is necessary to compensate for day to day variations in the electronic characteristics of the mass spectrometer. A value of 136.20 was selected as the isotopic ratio of tank nitrogen and all the experimental data were normalized to this value.

In this investigation the isotope effects were computed from the confidence intervals for combined analyses for the products of complete and partial reaction.

To compute the isotope effect the pooled estimate of the standard deviation for the combined experiments,

$$s_p = \frac{\left(\sum_{i\gamma} x_{i\gamma}^2 - \sum_{i=1}^{r} T_{i_1}^2/N_{i_1} - \sum_{i=1}^{r} T_{i_2}^{\;2}/N_{i_2}\right)^{1/2}}{k}, \quad (95)$$

and the associated number of degrees of freedom,

$$k = \sum_{i=1}^{r} (N_{i_1} + N_{i_2}) - 2r , \quad (96)$$

are required for each population. Let k and s_p be the number of degrees of freedom and the pooled estimate of the standard deviation for the product from complete reaction. Similarly let k' and s_p' represent the same quantities for the product from partial reaction. Using these values, the quantities k" and $s_p"$ are computed from the equations

$$k" = k + k' \quad (97)$$

and

$$k"(s_p")^2 = k(s_p)^2 + k'(s_p')^2 \quad (98)$$

The new interval is given by

$$Y - Y' \pm t_{k'', 0.025} \cdot s_{p''} \sqrt{1/\sum_{i=1}^{r} b_i + 1/\sum_{i=1}^{r} b_i'} \qquad (99)$$

where Y and Y' are the combined confidence intervals of the completely and partially reacted material, respectively, and $b_i = N_{i_1} \cdot N_{i_2} / (N_{i_1} + N_{i_2})$. The isotope effect is obtained by adding one to the above result and dividing by the normalizing factor, 136.20, i.e.,

$$\frac{k_{14}}{k_{15}} = \frac{1 + (Y - Y')}{136.20} \pm \frac{t_{k'', 0.025}}{136.20} \cdot s_{p''} \cdot \sqrt{1/\sum_{i=1}^{r} b_i + 1/\sum_{i=1}^{r} b_i'} \qquad (100)$$

The true value of the ratio of the rate constants can be expected to lie within these limits 95 per cent of the time.

In the computation of the confidence intervals for individual sample analyses it is convenient to adopt a sign convention whereby the intervals are considered as positive or negative quantities depending on the value of the 28 29 ratios relative to tank nitrogen (see page 129). When computing the isotope effect, however, the combined confidence intervals must always be taken as positive numbers.

The computation of isotope effects indirectly from confidence intervals is equivalent to the calculation of these effects directly from isotopic ratios. The equivalence of the two methods is demonstrated by the following considerations.

In the analysis of a sample prepared from the completely reacted quaternary ammonium salt, let a and a + x be the average values of the isotopic ratios of the tank and sample nitrogen, respectively. Similarly, in the analysis of a sample from partially reacted material let b and b + x represent the average values of the tank and sample isotopic ratios, respectively. If the value to which all samples are normalized is designated as N.F., the normalized values for complete and partial reactions are given by

$$\text{N.F.} \times \left(\frac{a + x}{a}\right) \tag{101}$$

and

$$\text{N.F.} \times \left(\frac{b + y}{b}\right) \tag{102}$$

respectively. The isotope effect may be computed as

$$\frac{k_{14}}{k_{15}} = \frac{\text{N.F.} \times \frac{(b + y)}{b}}{\text{N.F.} \times \frac{(a + x)}{a}} \tag{103}$$

$$= \frac{\frac{(b + y)}{b}}{\frac{(a + x)}{a}} \tag{104}$$

$$= \frac{ab + ay}{ab + ax} \tag{105}$$

$$= \frac{1 + \frac{y}{b}}{1 + \frac{x}{a}} \tag{106}$$

Using the binomial expansion of $(1 + x/a)^{-1}$ the value of k_{14}/k_{15} may be expressed as

$$\frac{k_{14}}{k_{15}} \cong \frac{(1 + \frac{y}{b})}{(1 - \frac{x}{a})} \qquad (107)$$

If the xy/ab term is neglected, equation (107) becomes

$$\frac{k_{14}}{k_{15}} \cong 1 + \frac{y}{b} - \frac{x}{a} \qquad (108)$$

An equivalent expression for the isotope effect can be obtained from the mid-points, θ, of the two confidence intervals. If (-x) and (-y) are the mid-points of the complete and partial reactions, respectively, the isotope effect is given by

$$\frac{k_{14}}{k_{15}} = 1 + \frac{y - x}{N.F.} \qquad (109)$$

$$= 1 + \frac{y}{N.F.} - \frac{x}{N.F.} \qquad (110)$$

$$\cong 1 + \frac{y}{b} - \frac{x}{a} \qquad (111)$$

which is equivalent to equation (108).

RESULTS

The per cent reaction of amine with halide, the recovery of ammonia from the Kjeldahl digestions and the 95 per cent confidence intervals computed from the mass spectrometer data are tabulated for the reaction systems studied. Each complete and partial reaction of an amine with an alkyl halide, with the exception of the reaction between triethylamine and isopropyl iodide, was performed in duplicate (see Table VI). Two or more nitrogen samples were prepared from the product of each quaternary ammonium salt reaction. The only exception was for the product of one of the partial reactions of triethylamine with ethyl iodide at 6°C. from which only one nitrogen sample was prepared.

The analytical results are tabulated as 95 per cent confidence intervals (equation 92) for the indirect analyses of each sample relative to tank nitrogen and for the intercomparisons between samples, i.e., the direct analyses of samples relative to one another. The results of repeated analyses of an individual sample were combined and expressed as a single confidence interval, namely, the confidence interval for combined analyses (equation 95). Since variations in the isotope effect arising from different extents of reaction at the same reaction temperature were too small to be measured, the results from all quaternary salts prepared at the same temperature were also combined and expressed as confidence intervals for combined analyses.

It will be noted that the confidence intervals are expressed as positive numbers for the nitrogen samples from all the reaction systems except the trimethylamine - ethyl bromide reaction where they are given as negative numbers. When the confidence intervals are expressed as positive numbers it indicates that the 28/29 ratios of the sample nitrogen, $\bar{X}_2$, were less than the isotopic ratios of tank nitrogen, $\bar{X}_1$. The 28/29 ratios of the nitrogen prepared from ethyl trimethylammonium bromide, however, were greater than the isotopic ratios of the tank nitrogen, i.e., $\bar{X}_1 < \bar{X}_2$; consequently, these confidence intervals are given as negative numbers.

Finally, isotope effects were computed from the confidence intervals for combined analyses using equation (96).

The Triethylamine - Ethyl Iodide Reaction

All nitrogen samples prepared from tetraethylammonium iodide formed by complete and partial reactions of triethylamine with ethyl iodide at 6.0, 25.0, 56.8 and 78.2°C. were first analyzed relative to tank nitrogen. The 95 per cent confidence intervals computed for the individual analyses and for the combined analyses are given in Table XIV.

TABLE XIV

CONFIDENCE INTERVALS FOR THE REACTION OF TRIETHYLAMINE WITH ETHYL IODIDE

Sample No.	Per cent Reaction %	Reac'n Temp. °C.	Kjeldahl Recovery %	95% Confidence Interval	
				Individual Analysis	Combined Analyses
COMPLETE REACTION					
B1-1	100.3	60	97.1	0.884±0.175	0.884±0.175
-2			98.5	0.753±0.125	0.753±0.125
B2-1	99.9	60	95.0	0.877±0.164	0.877±0.164
-2			96.7	0.914±0.195	
				0.968±0.201	
				0.926±0.248	
				0.996±0.173	0.951±0.098
-3			98.2	0.807±0.171	0.807±0.171
					0.87 ±0.06
PARTIAL REACTIONS					
1) BA1-1	2.3	6.0	97.0	0.814±0.144	0.814±0.144
BA2-1	4.7	6.0	94.7	0.853±0.127	0.853±0.127
-2			90.8*	0.794±0.160	0.794±0.160
-3			98.2	0.691±0.116	0.691±0.116
-4			94.6	0.739±0.145	0.739±0.145
					0.78 ±0.06
2) BB1-1	2.1	25.0	94.9	0.779±0.175	
				0.692±0.142	0.728±0.108
-2			98.1	0.537±0.130	0.537±0.130
-3			99.3	0.694±0.156	0.694±0.156
-4			97.9	0.537±0.140	0.537±0.140

(Cont'd)

TABLE XIV (Cont'd)

Sample No.	Per cent Reaction %	Reac'n Temp. °C.	Kjeldahl Recovery %	95% Confidence Interval Individual Analysis	95% Confidence Interval Combined Analyses
BB2-1	4.1	25.0	94.8	0.794±0.115	
				0.686±0.191	0.753±0.100
-2			97.6	0.667±0.184	0.667±0.184
-3			98.1	0.663±0.149	0.663±0.149
-4			96.6	0.678±0.132	0.678±0.132
					0.67 ±0.05
3) BC1-1	5.4	56.8	97.5	0.460±0.141	0.460±0.141
-2			96.5	0.627±0.142	0.627±0.142
-3			94.5	0.677±0.157	
				0.860±0.254	0.744±0.133
-4			95.6	0.510±0.221	
				0.724±0.121	
				0.670±0.143	0.662±0.085
BC2-1	6.6	56.8	97.8	0.538±0.110	0.538±0.110
-2			94.1	0.623±0.130	0.623±0.130
-3			93.7	0.760±0.202	
				0.678±0.140	0.707±0.112
-4			97.8	0.613±0.129	0.613±0.129
					0.65 ±0.04
4) BD1-1	6.4	78.2	95.2	0.552±0.143	0.552±0.143
-2			97.4	0.653±0.122	0.653±0.122
-3			95.4	0.677±0.141	
				0.651±0.130	0.665±0.095
BD2-1	8.6	78.2	97.5	0.487±0.128	0.487±0.128
-2			98.6	0.597±0.153	0.597±0.153
					0.60 ±0.05

*) The low recovery of ammonia from the Kjeldahl digestion of sample BA2-2 was due to mechanical loss during the distillation.

Isotope effects, k_{14}/k_{15}, computed from the confidence intervals for the combined analyses given in Table XIV are listed in Table XV.

TABLE XV

NITROGEN ISOTOPE EFFECTS FOR THE REACTION OF TRIETHYLAMINE WITH ETHYL IODIDE

Reaction Temp. (°C.)	k_{14}/k_{15}
6.0	1.0007 ±0.0006
25.0	1.0015 ±0.0006
56.8	1.0017 ±0.0006
78.2	1.0020 ±0.0006

From Table XV it can be seen that the value of k_{14}/k_{15} for the reaction between triethylamine and ethyl iodide increases slowly with increasing reaction temperature. The largest difference in the values of k_{14}/k_{15} occurs at the extremes of the reaction temperatures studied, i.e., 6 and 78°C. For these temperatures the limits of the values of k_{14}/k_{15} at the 95 per cent confidence level are 1.0001-1.0013 and 1.0014-1.0026, respectively. While the values do not overlap, the significance of this small difference might be questioned.

The significance of the temperature effect can be tested by taking the data from the partial reaction at 6°C. as a reference standard to which the isotopic ratios for the products of partial reaction are compared. This permits the calculation of $(k_{14}/k_{15})_t/(k_{14}/k_{15})_{6^oC.}$, the ratio of the isotope effect at temperature t to that at 6°C., without reference to the isotopic ratio for the product of complete reaction.* The $(k_{14}/k_{15})_t/(k_{14}/k_{15})_{6^oC.}$ ratios evaluated using equation (96) are tabulated in Table XVI.

The significance of the temperature effect can be further tested by performing a Student t-test on the data from each pair of partial reactions using an extension of equation (83); the calculated value of t is compared with $t_{\nu\alpha}$ where ν is the number of degrees of freedom and α is the significance level. The values of the Student t calculated from the data for each pair of partial reactions and the values of t_{ν} for α values of 0.05, 0.025 and 0.005 are also given in Table XVI.

---

* The quantity, $(k_{14}/k_{15})_t\ /(k_{14}/k_{15})_{6^oC.}$, may of course be calculated from the k_{14}/k_{15} values given in Table XIV. The uncertainty limits, however, will be larger because of the uncertainty in the isotopic ratios for the product of complete reaction which are used in calculating the k_{14}/k_{15} values.

TABLE XVI

NITROGEN ISOTOPE EFFECT RATIOS RELATIVE TO PARTIAL REACTION AT 6°C.

Reaction Temp., (°C.)	Student t				$\frac{(k_{14}/k_{15})_t}{(k_{14}/k_{15})_{6°C.}}$
	Calc'd	$t_{\nu,0.05}$	$t_{\nu,0.025}$	$t_{\nu,0.005}$	
25	3.300	1.964	2.247	2.816	1.0008±0.0006
56	3.863	1.963	2.245	2.814	1.0010±0.0006
78	4.829	1.964	2.247	2.818	1.0013±0.0006

In all cases the value of $(k_{14}/k_{15})_t/(k_{14}/k_{15})_{6°C.}$ is greater than unity and it increases slowly as the temperature of the reaction system is raised. Further, in every case, the calculated value of the Student t is greater than $t_{\nu\alpha}$, even at the one per cent significance level, $t_{\nu,\,0.005}$. This result shows that there are significant differences between the isotopic ratios of the products formed by partial reaction at temperatures t and 6°C. It is concluded, therefore, that the temperature dependence of k_{14}/k_{15} indicated in Table XV is real, that is, the isotope effect does increase slightly as the temperature of the reaction system is raised.

The relationship between the isotope effect and the reaction temperature was also studied by intercomparing the samples themselves. The 95 per cent confidence intervals computed from the intercomparison of nitrogen samples from tetraethylammonium iodide prepared under different experimental conditions are tabulated in Table XVII. The asteriks denote intercomparisons which did not meet the prescribed F-test and Student t-test at the 95 per cent level of significance.

The intercomparisons of the nitrogen from a partial reaction with that from the complete reaction give k_{14}/k_{15}, while the intercomparisons between the products of two partial reactions prepared at different temperatures give $(k_{14}/k_{15})_{t'}/(k_{14}/k_{15})_t$, the ratio of the isotope effects at temperatures t and t', where t' is the higher of the two temperatures. These values are shown in Table XVIII.

The isotope effects and isotope effect ratios obtained from the direct comparison of samples (Table XVIII) are less reliable than those obtained from the analysis of samples relative to tank nitrogen (Tables XV and XVI). The former are based on a smaller number of observations and, since very small differences were being measured, the errors associated with the mass spectrometer measurements are greater than in the sample-tank analyses. It will be noted that two of the intercomparisons did not pass the F-test and the Student t-test at the 95 per cent level of significance, which indicated that

TABLE XVII

CONFIDENCE INTERVALS FROM INTERCOMPARISONS OF TETRAETHYLAMMONIUM IODIDE SAMPLES

Samples Compared	Reaction Temperatures Compared	95% Confidence Interval
BA2-3 Vs B2-3	Partial at 6°C. Vs Complete Reaction	0.16±0.11
BB2-2 Vs B2-1	Partial at 25°C. Vs Complete Reaction	0.30±0.12
BB1-2 Vs BA1-1	Partial at 25°C. Vs Partial at 6°C.	0.32±0.11
BC1-1 Vs BA2-1	Partial at 56°C. Vs Partial at 6°C.	0.35±0.12
BD2-1 Vs BA2-4	Partial at 78°C. Vs Partial at 6°C.	0.28±0.15
BC2-1 Vs BB1-1	Partial at 56°C. Vs Partial at 25°C.	0.27±0.12
BC2-4 Vs BB2-3	Partial at 56°C. Vs Partial at 25°C.	0.17±0.14 *
BD1-3 Vs BB2-4	Partial at 78°C. Vs Partial at 25°C.	0.23± .19
BD1-1 Vs BB2-3	Partial at 78°C. Vs Partial at 25°C.	0.03±0.15*

* Failed F-test and Student t-test.

TABLE XVIII

NITROGEN ISOTOPE EFFECTS AND RATIOS FROM INTERCOMPARISONS OF TETRAETHYLAMMONIUM IODIDE SAMPLES

Samples Intercompared	k14/k15	$\frac{(k14/k15)_{t'}}{(k14/k15)_{t}}$
Partial at 6°C Vs Complete Reaction	1.0012 ± 0.0008	
Partial at 25°C Vs Complete Reaction	1.0022 ± 0.0009	
Partial Reactions 25°C Vs 6°C		1.0023 ± 0.0008
Partial Reactions 56°C Vs 6°C		1.0026 ± 0.0009
Partial Reactions 78°C Vs 6°C		1.0021 ± 0.0011
Partial Reactions 56°C Vs 25°C		1.0020 ± 0.0009 1.0013 ± 0.0010*
Partial Reactions 78°C Vs 25°C		1.0017 ± 0.0014 1.0002 ± 0.0010*

* Failed F test and Student t-test.

these samples did not differ at the chosen level of significance. Even in these cases, however, the values of $(k_{14}/k_{15})_{t'}/(k_{14}/k_{15})_t$ are greater than unity.

The isotope effects, k_{14}/k_{15}, obtained by intercomparison of samples are in substantial agreement with the effects observed at the corresponding temperatures by the sample-tank analyses (Table XV). Although the intercomparison of partial reactions establish that the value of k_{14}/k_{15} is lower at 6°C. than at all the higher temperatures, these results do not show a temperature trend at the higher reaction temperatures. The reader is reminded that the errors involved in the computation of isotope effect ratios from intercomparison data are considerably larger than in the calculations based on sample-tank measurements. When these errors are considered, the absence of a clearly defined temperature dependence is not surprising.

In summary, the experimental data from the analyses of samples relative to tank nitrogen and from the intercomparisons between samples both clearly indicate that the nitrogen isotope effect, k_{14}/k_{15}, is slightly greater than unity in the bond formation reaction of triethylamine with ethyl iodide. Further, the results from the sample-tank analyses indicate that the value of the isotope effect increases slightly as the temperature of the reaction is raised.

The Trimethylamine - Ethyl Bromide Reaction

The 95 per cent confidence intervals computed from the analyses of nitrogen samples prepared from ethyltrimethylammonium bromide relative to tank nitrogen are given in Table XIX.

Nitrogen from several of the quaternary ammonium salts formed by partial reaction were intercompared with the product of complete reaction. One intercomparison of nitrogen from ethyltrimethylammonium bromide formed by partial reaction at 25°C. with the product of partial reaction at 6°C. was attempted. The results of these intercomparisons are given in Table XX. It will be noted from this Table that the analytical data from all of the intercomparisons did not pass the F-test and the Student t-test at the prescribed levels of significance. Further, the results of the intercomparisons were not consistent with respect to the direction of isotopic enrichment. The negative sign before the confidence intervals indicates that the product of partial reaction was depleted in N^{14} relative to the product of complete reaction.

The isotope effects, k_{14}/k_{15}, computed from the analyses of samples relative to tank nitrogen are given in Table XXI.

TABLE XIX

CONFIDENCE INTERVALS FOR THE REACTION OF TRIMETHYLAMINE WITH ETHYL BROMIDE

Sample No.	Per Cent Reaction %	Reac'n Temp. °C.	Kjeldahl Recovery %	95% Confidence Interval Individual Analysis	 Combined Analyses
Complete Reactions					
D1-1	100.0	25	94.7	-0.475 ± 0.157	-0.475 ± 0.157
-2			97.7	-0.570 ± 0.139	-0.570 ± 0.139
D2-1	100	25	96.7	-0.541 ± 0.198	-0.541 ± 0.198
-2			97.5	-0.563 ± 0.115	-0.563 ± 0.115
					-0.54 ± 0.07
Partial Reactions					
(1) DA1-1	5.2	5.8	97.6	-0.457 ± 0.147	-0.457 ± 0.147
-2			97.5	-0.484 ± 0.126	-0.484 ± 0.126
-3			97.8	-0.484 ± 0.129	-0.484 ± 0.129
DA2-1	6.3	5.8	98.1	-0.455 ± 0.120	-0.455 ± 0.120
-2			96.6	-0.405 ± 0.122	-0.405 ± 0.122
					-0.45 ± 0.06
(2) DB1-1	4.4	25.2	98.4	-0.325 ± 0.153	-0.325 ± 0.153
-2			98.5	-0.340 ± 0.210	
				-0.409 ± 0.165	-0.378 ± 0.129
DB2-2	5.7	25.2	96.5	-0.445 ± 0.180	-0.445 ± 0.180
			100.0	-0.481 ± 0.115	-0.481 ± 0.115
					-0.41 ± 0.07

TABLE XX

CONFIDENCE INTERVALS FROM INTERCOMPARISONS BETWEEN ETHYLTRIMETHYLAMMONIUM BROMIDE SAMPLES

Samples Compared	Reaction Temperatures Compared	95% Confidence Interval
DB1-1 Vs D1-1	Partial at 25°C. Vs Complete Reaction	+ 0.08 ± 0.17*
DB1-1 Vs D1-2	Partial at 25°C. Vs Complete Reaction	- 0.07 ± 0.15*
DB1-2 Vs D2-1	Partial at 25°C. Vs Complete Reaction	- 0.13 ± 0.15*
DB2-2 Vs D2-2	Partial at 25°C. Vs Complete Reaction	- 0.12 ± 0.15*
DB1-2 Vs DA2-1	Partial at 25°C. Vs Partial at 6°C.	- 0.02 ± 0.18*

* Failed F-test and Student t-test.

TABLE XXI

NITROGEN ISOTOPE EFFECTS FOR THE REACTION OF TRIMETHYLAMINE WITH ETHYL BROMIDE

Reaction Temp., (°C.)	k_{14}/k_{15}
6	0.9994 ±0.0006
25	0.9991 ±0.0007

No attempt was made to compute k_{14}/k_{15} from the intercomparison data.

From the results of the sample - tank analyses and the intercomparisons between samples it is evident that the nitrogen isotope effect in the reaction of trimethylamine with ethyl bromide is extremely small, i.e., the value of k_{14}/k_{15} is effectively unity at both 6 and 25°C. It will be noted that the values of k_{14}/k_{15} computed from the sample - tank analyses are slightly less than unity. No significant difference, however, was observed when ethyltrimethylammonium bromide formed by partial reaction was analyzed relative to the product of complete reaction. Since the intercomparisons did not support the results obtained from the sample - tank analyses, it cannot be stated unequivocally that the value of k_{14}/k_{15} is significantly less than unity.

It should be emphasized that the failure of the intercomparison data to meet the F-test and the Student t-test at the prescribed levels of significance does not necessarily mean that the samples are identical. The result merely means that there is no difference between the samples at the chosen level of significance, i.e., $\alpha = 0.05$. A positive result might be obtained by selecting some other level of significance. i.e., $\alpha = 0.10$ or some higher value. All analytical data were examined by a uniform statistical procedure, and when data failed any of these tests it was concluded that there was no significant difference at that probability level.

The nitrogen isotope effect for the reaction of trimethylamine with ethyl bromide would appear to be slightly lower in value than that for the reaction of triethylamine with ethyl iodide at comparable temperatures. The values of k_{14}/k_{15} at a reaction temperature of 6°C. are 0.9994 ±0.0006 and 1.0007 ±0.0006 for the trimethylamine - ethyl bromide and triethylamine - ethyl iodide reactions, respectively. The difference seems to be even greater for a reaction temperature of 25°C. i.e., 0.9991 ±0.0007 for the reaction between trimethylamine and ethyl bromide and 1.0015 ±0.0006 for the reaction of triethylamine with ethyl iodide.

The Triethylamine - Methyl Iodide Reaction

The 95 per cent confidence intervals computed from the mass spectrometer data obtained for the complete and partial reactions of triethylamine with methyl iodide are given in Table XXII.

The nitrogen isotope effect for this bond formation reaction, calculated from the data in Table XXII, was found to be 1.0009 ±0.0005 at 8.2°C. This value is identical at this level of significance with that observed for the reaction of triethylamine with ethyl iodide at 6°C., i.e., k_{14}/k_{15} = 1.0007 ±0.0006.

The activation energies of the triethylamine - methyl iodide and the triethylamine - ethyl iodide reactions differ by approximately two kilocalories (see page 63). Since the isotope effects are identical within the experimental error, there is no apparent relationship between the magnitude of the isotope effect and the activation energy of the reaction over this limited range of activation energies. For reaction systems exhibiting such very small isotope effects this result perhaps is to be expected.

The Triethylamine - Isopropyl Iodide Reaction

Only one partial reaction of triethylamine with isopropyl iodide was performed. The nitrogen samples prepared from the isopropyltriethylammonium iodide formed by partial

TABLE XXII

CONFIDENCE INTERVALS FOR THE REACTION OF TRIETHYLAMINE WITH METHYL IODIDE

Sample No.	Per Cent Reaction %	Reac'n Temp. °C.	Kjeldahl Recovery %	95% Confidence Intervals Individual Analysis	95% Confidence Intervals Combined Analyses
Complete Reactions					
A1-1	100.0	25	97.6	0.842 ± 0.180	0.842 ± 0.180
-2			97.9	0.829 ± 0.127	
				1.069 ± 0.194	0.925 ± 0.106
A2-1	100	25	97.8	0.918 ± 0.116	0.918 ± 0.116
-2			96.5	0.916 ± 0.122	0.916 ± 0.122
-3			97.8	0.775 ± 0.113	0.775 ± 0.113
					0.87 ± 0.05
Partial Reactions					
AA1-1	4.1	8.2	97.5	0.772 ± 0.133	0.772 ± 0.133
-2			99.2	0.723 ± 0.140	0.723 ± 0.140
-3			96.6	0.849 ± 0.153	0.849 ± 0.153
AA2-1	7.4	8.2	96.6	0.821 ± 0.147	
				0.634 ± 0.277	
				0.743 ± 0.176	0.759 ± 0.106
-2			97.5	0.771 ± 0.181	0.771 ± 0.181
-3			96.1	0.693 ± 0.113	0.693 ± 0.113
					0.76 ± 0.05

reaction were analyzed relative to tank nitrogen. The 95 per cent confidence intervals for these analyses are tabulated in Table XXIII.

TABLE XXIII

CONFIDENCE INTERVALS FOR THE REACTION OF TRIETHYLAMINE WITH ISOPROPYL IODIDE

Sample No.	Per Cent Reaction	Reaction Temp., °C.	Kjeldahl Recovery, %	95% Confidence Intervals
Cl-1	2.4	61	93.5	1.246 ± 0.190
Cl-2	2.4	61	94.5	1.138 ± 0.158
				1.19 ± 0.12

Since the reaction of triethylamine with isopropyl iodide is very slow, no attempt was made to prepare the product of complete reaction. The isopropyltriethylammonium iodide was prepared from triethylamine of the same isotopic composition as that used in the preparation of tetraethylammonium iodide. Therefore, the confidence intervals for the product of the complete reaction of triethylamine with ethyl iodide could be used to compute the isotope effect for the reaction between triethylamine and isopropyl iodide. The apparent nitrogen isotope effect k_{14}/k_{15}, for this reaction was found to be 0.9978 ± 0.0009 at 61°C., i.e., the quaternary ammonium salt recovered from the reaction mixture was enriched with the N^{15} isotope.

The values of k_{14}/k_{15} observed for the unidirectional bond formation reactions included in this investigation are all close to unity and in two of the reaction systems are slightly greater than unity. For the reaction of triethylamine with isopropyl iodide, however, the apparent value of k_{14}/k_{15} is significantly less than unity. It is conceivable that the observed isotope effect could result from a larger transition state term for the reaction system triethylamine - isopropyl iodide. One might expect, however, that the reverse would be true since the steric hinderance in the isopropyl iodide molecule is greater than in methyl iodide or ethyl iodide. This would be expected to lead to a smaller transition state term and a larger positive value for k_{14}/k_{15}, which clearly is not the case.

Observations made during the preparation of isopropyltriethylammonium iodide (see page 70) clearly indicate that the reaction of triethylamine with isopropyl iodide is actually a complex system in which both a bond formation process and an elimination side reaction occur. In the elimination reaction the quaternary iodide is attacked by unreacted triethylamine with the resultant formation of propylene and triethylammonium iodide. The occurrence of this decomposition reaction, in which C - N bonds are ruptured, suggests an alternative explanation for the apparent "reverse" isotope effect observed in this reaction system. It would be anticipated that the isotope effect would be small in the bond formation process but an appreciable effect could occur in the elimination reaction. In the decomposition of isopropyltriethylammonium iodide it would be expected that C-N^{14} bonds would be broken at a faster rate than C-N^{15} bonds. Since the quaternary ammonium iodide is formed more rapidly then it is decomposed, this elimination reaction could result in an enrichment of the N^{15} isotope in the undecomposed isopropyltriethylammonium iodide. Therefore, the apparent "reverse" isotope effect can be readily explained in terms of an elimination side reaction.

A true k_{14}/k_{15} value cannot be computed from the isotopic ratios as there are a number of unknown factors, not the least of which is the extent of decomposition. A rough calculation based on the ammonia recovered from the Kjeldahl digestions indicates that the decomposition had proceeded to approximately 20 per cent. If the isotopic fractionation observed in the reaction of isopropyl iodide with triethylamine is indeed due to decomposition of the quaternary salt, this must mean that there is a fairly large isotope effect associated with the elimination reaction. This is in contrast to the small isotope effect reported by Saunders and Asperger for the decomposition of 2-phenylethyldimethylsulphonium bromide (65). The apparent difference between these two elimination reactions would not be surprising since the βhydrogen in the 2-phenylethyldimethylsulphonium ion would be much more acidic because of the presence of the phenyl group on the same carbon and as a result, C-N bond rupture would have proceeded to a much smaller extent in the transition state.

DISCUSSION

Qualitative Predictions of the Bigeleisen Theory

The Bigeleisen equation for a simple bond formation or bond rupture process is given by

$$\frac{k_1}{k_2} = \left(\frac{m_2^*}{m_1^*}\right)^{\frac{1}{2}} \left[1 + \sum_i^{3n-6} G(u_i)\,\Delta u_i - \sum_i^{3n'-6} G(u_i^{\neq})\,\Delta u_i^{\neq}\right] \qquad (1)$$

where the subscripts 1 and 2 refer to the light and heavy isotopes, respectively, the symbol $\neq$ refers to the activated complex, k is the rate constant, m^* is the effective mass of the molecule along the reaction coordinate (6). The square bracket term describes the free energy effects associated with the initial state and the activated complex. G(u) is a funcdefined by Bigeleisen and Mayer (7) and

$$\Delta u_i = \frac{hc}{kT}\,(w_{1i} - w_{2i}) \qquad (33)$$

where w is the fundamental vibration frequency. The effective mass term is calculated as the reduced mass of an imaginary diatomic molecule whose bond is being broken or formed in the rate determining step (11).

In a simple bond formation process the isotopic atoms, between which the bond is being formed, are more "tightly bound" in the activated complex than in the initial state. Therefore, the free energy term for the activated state is greater than the free energy term for the initial state,

$$\sum_{i}^{3n'-6} G(u_i^{\neq})\Delta u_i^{\neq} > \sum_{i}^{3n-6} G(u_i)\Delta u_i.$$

Consequently, the square bracket term is less than unity and tends to cancel the effective mass term. Since the effective mass term and the free energy term are acting in opposite directions, the theory qualitatively predicts that the value of the ratio of isotopic rate constants will be less than the value of the effective mass term. The resultant value of k_1/k_2 depends primarily on the magnitude of the square bracket term. The Bigeleisen equation predicts only that the isotope effect will be small. It is unable to predict whether k_1/k_2 will be less or greater than unity.

Since the free energy terms of the Bigeleisen expression depend primarily on the values of Δu_i, which are related to temperature by the expression $\Delta u_i = hc(\omega_{1i} - \omega_{2i})/kT$, any isotope effect will be temperature dependent. As the temperature of the reaction system is raised, the magnitude of the free energy terms associated with the activated complex and the initial state decrease in value. In a bond formation reaction the free energy term for the activated complex is greater than the term for the initial state; therefore, as the reaction temperature is increased, there is less cancellation of the effective mass term and the ratio of isotopic rate constants, k_1/k_2, should increase and approach the value of the effective mass term as an upper limit. In a reaction involving bond rupture, however, the free energy term associated with the initial state term is greater than that for the activated complex, hence, the free energy term makes a smaller contribution to the final value of k_1/k_2 with increasing reaction temperature and the value of k_1/k_2 should decrease and approach the value of the effective mass term as a lower limit.

All terms in the Bigeleisen equation must be evaluated for an exact calculation of an isotope effect. However, the model must be further simplified before an estimate of the isotope effect can be made. In a bond formation process it is convenient to assume that the process can be treated as the formation of a hypothetical bond from separated atoms and that the vibrational frequencies of all bonds, other than the one being formed, are unchanged during the reaction. Since the isotopic atoms are assumed to be an infinite distance apart in the initial state

$$\sum_{i}^{3n'-6} G(u_i)\,\Delta u_i = 0$$

and equation (1) reduces to

$$\frac{k_1}{k_2} = \left(\frac{m_2^*}{m_1^*}\right)^{\frac{1}{2}} \left[1 - \sum_{i}^{3n'-6} G(u_i^{\neq}) \Delta u_i^{\neq}\right] \tag{49}$$

Finally, a configuration must be assumed for the activated complex. If it is assumed that the bond is completely formed in the activated complex, $\sum_{i}^{3n'-6} G(u_i^{\neq}) \Delta u_i^{\neq}$ can be evaluated from spectroscopic data and a value assigned to k_1/k_2. This estimate of k_1/k_2 will be less than the true value to the extent that the bond is not completely formed in the activated complex.

The Bigeleisen model postulates that the effective mass term is the same for all reactions involving the same isotopes, i.e., the reduced mass of the atoms across the bond being formed or broken. Variations in the magnitude of isotope effects, therefore, should arise only from differences in the free energy terms for the activated complexes and the initial states of the bonds undergoing reaction.

Quantitative Predictions of the Bigeleisen Theory

The ratio of the isotopic rate constants for the formation of a C-N bond, i.e., the reaction of an amine with a halide, can be computed using the simplified model, equation (49).

If the effective masses are taken as the reduced masses of the atoms which are forming the bond the effective mass term is given by

$$\left(\frac{m_2^*}{m_1^*}\right)^{\frac{1}{2}} = \frac{\mu_{(C^{12} - N^{15})}}{\mu_{(C^{12} - N^{14})}}$$

$$= 1.0158$$

The fundamental vibration frequency for the C - N bond in the tetramethylammonium ion is 752 cm^{-1} (66). If this value is taken as the wave number for the $C^{12} - N^{14}$ bond in the activated complex, $\omega_1^{\neq}$, then the wave number for the $C^{12} - N^{15}$ bond in the activated complex, $\omega_2^{\neq}$, is computed as

$$\omega_2^{\neq} = \omega_1^{\neq} \left(\frac{m_1^*}{m_2^*}\right)^{\frac{1}{2}} = 740 \text{ cm}^{-1}$$

$u_1^{\neq}$ and $\Delta u_1^{\neq}$ are computed from the formulae

$$u_1^{\neq} = \frac{hc\,\omega_1^{\neq}}{kT} \qquad (31)$$

$$\Delta u_1^{\neq} = \frac{hc\,(\omega_1^{\neq} - \omega_2^{\neq})}{kT} \qquad (33)$$

The function G(u) defined as

$$G(u) = \frac{1}{2} - \frac{1}{u} + \frac{1}{e^u - 1} \qquad (36)$$

is then computed and used to evaluate $\sum_i^{3n'-6} G(u_i^{\neq})\,\Delta u_i^{\neq}$, the free energy term for the activated complex. This calculation can be simplified by using the Table of G(u) values published by Bigeleisen and Mayer (7).

From these quantities values of k_{14}/k_{15} are computed for the reaction temperatures investigated. These calculated values of k_{14}/k_{15} are compared with the experimental values in Table XXIV. Only the results from the analyses of samples relative to tank nitrogen are given in this table as they provide the best estimates of the isotope effects.

TABLE XXIV

THEORETICAL AND EXPERIMENTAL VALUES OF k_{14}/k_{15}

Reaction Temp., (°C.)	k_{14}/k_{15}			
	Experimental			
	Et_3N + MeI	Et_3N + EtI	Et_3N + EtBr	Calculated
6 - 8	1.0009 ±0.0005	1.0007 ±0.0006	0.9994 ±0.0007	0.9993
25.0		1.0015 ±0.0006	0.9991 ±0.0007	1.0010
56.8		1.0017 ±0.0006		1.0034
78.2		1.0020 ±0.0006		1.0046

From this table it can be seen that the isotope effects calculated using the simplified model are very close to unity and of the same order of magnitude as was observed experimentally. Quantitatively, however, the agreement is only fair and is particularly poor at the extremes ends of the temperature range studied. The temperature dependence of the calculated effect, although in the same direction as that observed experimentally, is much larger than the dependence found.

When the extreme assumptions inherent in these calculations are considered, the absence of quantitative agreement between theory and experiment is not surprising. The procedures followed in estimating the effective mass term and the free energy term are both open to question.

Apart from the assumptions inherent in absolute rate theory further drastic simplifications must be made in the Bigeleisen model before the free energy term of the activated complex can be estimated. The assumption that the other bonds in the reacting molecules are unaffected during the bond formation process and that the reaction can be considered as merely the formation of a diatomic molecule from separated atoms is an extreme simplification. Furthermore, an extreme model, namely that of complete bond formation, has been taken for the activated complex. To the extent that the bonds are less than completely formed, the free energy term so calculated is too large. This would have the further consequence of giving too large a temperature dependence to the calculated effect since the higher the frequency for the C-N bond in the activated complex the higher the value of $(\omega_{1i} - \omega_{2i})$ in equation (33). As noted earlier, the temperature dependence of the calculated effect is considerably greater than that observed experimentally.

On the basis of these considerations it seems reasonable to conclude that the calculated values for the free energy term associated with the activated complex are too large. In spite of this, however, there is fair agreement between the calculated and experimental values for the k_{14}/k_{15} ratios. This would suggest that calculation of the mass term as the reduced mass of a hypothetical C-N bond also leads to a value which is too high. As can be seen from Equation (49), the mass and free energy terms operate in opposite directions in a bond formation process and if calculated values for both terms are too large there could be fortuitous cancellation resulting in calculated isotope effects in fair agreement with the experimental values.

The assumption that the effective mass term can be treated as the reduced mass of a hypothetical diatomic molecule between which a bond is broken or formed is based on a derivation developed by Slater (11). This model has given results in good agreement with experiment when applied to simple bond rupture reactions. The validity of this treatment for bond formation, however, has been questioned by others in this laboratory (67). According to the Bigeleisen model the isotope effect in a bond formation process should increase with reaction temperature to the value of the effective mass term as an upper limit. If the effective mass term is calculated as the reduced mass of a hypothetical C-N molecule, the isotope effect should tend to a value of 1.0158. In the reaction of triethylamine with ethyl iodide, however, it was found that the isotope effect appeared to be levelling off at 1.002, a value far below the predicted value, 1.0158. This fact prompted speculation as to the correct form for the effective mass term. In particular, it raised the question as to whether the masses of the reacting molecules should be considered in the evaluation of this term.

The Slater theorem is based upon his classical investigation of unimolecular reaction rates at high pressures. The model used assumes that the molecule decomposes when a given coordinate, q, reaches a critical extension, q_0. This leads to an expression for the rate constant

$$k = \gamma e^{-E_0/kT} \qquad (112)$$

where γ may be defined as the ratio of all 3n-6 frequencies of the normal molecule to 3n-7 frequencies obtained when no motion is permitted along q. For the case where q is just the extension between two atoms A and B, the isotope effect is found to be quite generally

$$\frac{k_1}{k_2} = \left(\frac{\mu_2}{\mu_1}\right)^{\frac{1}{2}} e^{-(E_0^1 - E_0^2)/kT} \qquad (113)$$

where $\mu = m_A^1 m_B / m_A^2 m_B$. The frequency factor in this classical equation behaves isotopically as though one were dealing with a diatomic molecule of two atoms, the bond between which is being broken.

In a forthcoming publication Bigeleisen points out that in regarding Slater's coordinate, q, as the mode of decomposition in the transition state method, one must examine the potential energy surface (68). The decomposition path involves passage over the lowest possible barrier between reactants and products. Bigeleisen suggests that it is perhaps unreasonable to assume that this path necessarily involves only motion of the atoms A and B of the activated complex. Instead, a more reasonable formulation of the simple decomposition process would be to choose q as the coordinate which tears the decomposition fragments apart. Such a coordinate would lead "roughly" to the relation

$$\frac{\nu^{\neq}_{1L}}{\nu^{\neq}_{2L}} = \left[\left(\frac{1}{M_{\alpha 1}} + \frac{1}{M_{\beta 1}}\right) \Big/ \left(\frac{1}{M_{\alpha 2}} + \frac{1}{M_{\beta 2}}\right)\right]^{\frac{1}{2}} \qquad (114)$$

where M_α and M_β are the masses of the two fragments of the decomposition. Since the Slater and the transition state models are quite different they can lead to different results. Bigeleisen points out that the use of the Slater function, $(\mu_2/\mu_1)^{\frac{1}{2}}$, as the temperature independent factor does not obviously follow from a consideration of the potential energy surface and thus its use must be regarded as an intermingling of the transition state and Slater approaches.

Similar arguments can be applied to the case of simple bond formation. If these arguments are valid it would be anticipated that the effective mass term computed as the reduced mass of a hypothetical diatomic molecule would be considerably larger then the true value. Further, the Slater method leads to the same value of the effective mass term for all reactions between homologous reactants. On the other hand the effective mass terms computed by the fragment method vary with the reaction system since the masses of the reacting fragments or molecules enter into the calculations. The Slater and the fragment methods of computing the effective mass terms for the reaction systems included in this investigation are compared in Table XXV.

TABLE XXV

VALUES OF EFFECTIVE MASS TERMS

	Effective Mass	
Reaction System	Slater Method	Fragment Method
$Et_3N + MeI$	1.0158	1.0029
$Et_3N + EtI$	1.0158	1.0030
$Me_3N + EtBr$	1.0158	1.0054

It is difficult to explain the results from the reaction of triethylamine with ethyl iodide in terms of the Bigeleisen model for simple bond formation. As pointed out earlier the very small temperature dependence of the observed isotope effect compared to that obtained by calculation suggests that the free energy term used in the calculation of k_{14}/k_{15} is too large. Despite the extreme model used, however, the agreement between experiment and theory is reasonably good. This apparent agreement suggests that the effective mass term also is too large. Finally, the theory predicts that the value of k_{14}/k_{15} should tend to that of the effective mass term as an upper limit with increasing reaction temperature. While the experiments did not cover an extended temperature range, the isotope effect seems to be levelling off at a value, 1.002, very close to that calculated from the fragment model, 1.0029, and much below the value predicted by the hypothetical diatomic model, 1.0158. It is unfortunate that the benzene solvent did not permit measurement of the isotope effects over a wider temperature range. The isotope effects at temperatures below 0°C. would be particularly interesting.

The reaction of trimethylamine with ethyl bromide has been studied in an attempt to obtain additional information as to the correct form of the effective mass term. As shown in Table XXV the fragment model gives a mass term of 1.0054 for this reaction compared to a value of 1.0029 for the reaction of triethylamine with ethyl iodide. On the assumption that the free energy term for the activated complex is the same in the two reactions, one would expect then to observe a significantly higher isotope effect for the trimethylamine-ethyl bromide system if the mass term depends upon the masses of the reacting molecules and approximately the same isotope effect if this term depends on the reduced mass of the C - N bond only.

Unfortunately, there is no reason for believing that the free energy term for the activated complex would be the same in the two reaction systems. Indeed, for reasons which will be discussed later, one might expect that bond formation would proceed to a greater extent in the activated complex for the trimethylamine - ethyl bromide reaction. This would result in a higher free energy term for the activated complex, hence, a lower over-all free energy term for this reaction compared to that of the triethylamine - ethyl iodide reaction. This lower free energy would tend to compensate for any increased mass term. If, despite this, a larger value of k_{14}/k_{15} is observed for the reaction of trimethylamine with ethyl bromide, this would provide support for the fragment model. However, if the isotope effect for this reaction has the same value or is less than that observed for the reaction of triethylamine with ethyl iodide, no conclusion concerning the correct form for the mass term would be possible since a lower free energy term might more than compensate for a higher effective mass term.

The experimental values observed in the trimethylamine - ethyl bromide and the triethylamine - ethyl iodide reactions are compared in Table XXVI.

TABLE XXVI

k_{14}/k_{15} IN THE REACTIONS OF TRIMETHYLAMINE WITH ETHYL BROMIDE AND TRIETHYLAMINE WITH ETHYL IODIDE

Reaction Temp., (°C.)	k_{14}/k_{15}	
	Et_3N - EtI	Me_3N - EtBr
6	1.0007 ±0.0006	0.9994 ±0.0006
25	1.0015 ±0.0006	0.9991 ±0.0007

Since the values of k_{14}/k_{15} for the reaction of trimethylamine with ethyl bromide would appear to be less than the isotope effects observed for the reaction between triethylamine and ethyl iodide at the same reaction temperatures, this experiment failed to differentiate between the two models for the effective mass term. The lower isotope effect for the trimethylamine - ethyl bromide reaction indicates a higher free energy term for the activated complex of this reaction as compared to that of the triethylamine - ethyl iodide system. There are several reasons why the activated complex in the trimethylamine - ethyl bromide reaction should have a larger free energy term.

Considering first the two alkyl halides, the bromine atom is more electron attracting than the iodine atom and this tends to lower the electron density on the α-carbon of the halide, the site of nucleophilic attack by the amine. Since the electron density on the α-carbon is lower for ethyl bromide than for ethyl iodide, one might expect that the C - N bond would form to a greater extent in the activated complex leading to methyltriethylammonium bromide. Secondly, trimethylamine is a stronger base than triethylamine. This would result in a greater electron transfer in the activated complex of methyltriethylammonium bromide and a more tightly-bound transition state. Finally, Brown has demonstrated that trimethylamine undergoes complex formation more readily than triethylamine because of the smaller steric restrictions of the former (69). All three factors tend to promote a more tightly-bound activated complex for the trimethylamine - ethyl bromide system than for the triethylamine - ethyl iodide reaction system. This in turn results in a larger free energy term for the activated complex of ethyltrimethylammonium bromide and a greater cancellation of the effective mass term.

Conclusions

Although the isotope effects observed in the present investigation are very small and the effect of temperature and structure of the reactants on the magnitude of these effects is barely greater than the error limits of the mass spectrometer measurements, nevertheless, it has been possible to draw significant conclusions from the experimental results.

In the first place, it is clear that for the simple bond formation reactions studied that the mass and free energy factors effectively cancel each other with the result that the ratio of rate constants for the isotopic isomers is very close to unity. In terms of the Bigeleisen hypothesis, this means that the transition state, in so far as the nitrogen atom is concerned, is more "tightly bound" than the initial state. Indeed, one might consider that the observation of these very small effects for these bond formation reactions provides support for the Bigeleisen approach to the problem of isotope effects in unidirectional reactions.

Of perhaps greater significance is the observation that the magnitude of the isotope effect increases with increasing reaction temperature. This temperature dependence is predicted by the Bigeleisen equation for any system in which the free energy term for the transition state is greater than that for the initial state. Thus, the temperature dependence is in contrast to the temperature effect previously observed in bond rupture processes where the magnitude of the isotope effect decreases with increasing temperature.

Finally, the close agreement between the calculated and the observed isotope effects, despite the extreme model used in the calculation of the transition state term, the much lower temperature coefficient for the observed effect compared to that predicted on the basis of the assumed model, and the fact that the effect appears to level-off at a value well below that given by the Slater mass model all provide support for the fragment model recently suggested by Bigeleisen for the calculation of the effective mass term.

It is recognized that more precise mass spectrometer measurements would have been highly desirable in this investigation. It should be pointed out, however, that a more sensitive instrument would not necessarily have provided more accurate results since the chemical procedures involved in sample preparation might then have been the limiting factor. In spite of the limitations imposed by the mass spectrometer measurements, it has been possible, through repeated sample analyses and the application of rigorous statistical tests, to obtain what is believed to be the most precise nitrogen isotope effects reported to date.

SUMMARY

1. The nitrogen-15 isotope effects have been measured for the simple bond formation reactions in benzene solvent of triethylamine with methyl iodide, trimethylamine with ethyl bromide and triethylamine with ethyl iodide.

2. A nitrogen-15 isotope effect of 1.0007 ±0.0006 was found for the reaction of triethylamine with methyl iodide at 8°C.

3. The nitrogen-15 isotope effects for the reaction of trimethylamine with ethyl bromide have been measured at 6 and 25°C. For these temperatures the isotope effects were 0.9994 ±0.0006 and 0.9991 ±0.0007, respectively.

4. The nitrogen-15 isotope effects for the reaction of triethylamine with ethyl iodide have been measured at four temperatures. The isotope effects were found to be: 6°C., 1.0007 ±0.0006; 25°C., 1.0015 ±0.0006; 56.8°C. 1.0017 ±0.0006; 78.2°C., 1.0020 ±0.0006.

5. The mass spectrometer data were tested by rigorous statistical methods before being used to calculate isotope effects. The results for the reaction of triethylamine with ethyl iodide were further tested statistically to establish the significance of the temperature dependence of the isotope effect.

6. Tests have been performed to establish definitely that reactions of triethylamine with ethyl iodide and trimethylamine with ethyl bromide were strictly unidirectional under

the reaction conditions employed and that the chemical procedures used to convert the quaternary ammonium salts to molecular nitrogen proceeded quantitatively.

7. This investigation constitutes the first study of isotope effects in unidirectional bond formation reactions and is of special interest in that it provides a test of the Bigeleisen theory of isotope effects with respect to this type of chemical change.

8. The results have been discussed in terms of the Bigeleisen theory of isotope effects with special reference to the correct form for the effective mass term. The results of this investigation provide support for the fragment model recently proposed by Bigeleisen for the calculation of the effective mass term.

APPENDIX I

Statistical Analysis of Sample Data and the Computation of Confidence Intervals

Four representative examples of the calculations leading to 95 per cent confidence intervals are given to illustrate the mathematical procedure and the interpretation of the statistical results. In the first example, a tank-sample comparison, the data met the prescribed statistical tests and no observations were rejected. The scatter of the results in the second example, also a tank-sample comparison, was greater than permissible at the chosen level of significance, hence observations were rejected. In the third example, the data from an intercomparison passed the modified statistical procedure and a 95 per cent confidence interval was computed. The samples intercompared in the last example were not significantly different at the $\alpha = 0.05$ level.

Example 1: Sample BA1 - 1

The raw data on which the statistical analyses are based are the 28/29 mass ratios, $x_{1\gamma}$, of the two subgroups which are analyzed relative to each other. The results of the analysis of sample BA1 - 1 against tank nitrogen are given in Table 1a where the columns headed by the letters A and B contain the 28/29 ratios for tank and sample nitrogen, respectively.

Table 1a

MASS SPECTROMETER DATA

A_1	B_1	A_2	B_2	A_3	B_3	A_4
136.77	136.04	137.01	136.18	136.47	135.67	136.58
137.04	135.82	137.04	135.99	136.82	136.41	136.71
136.92	136.23	136.40	135.44	137.26	136.26	136.69
136.99	135.91	136.58	135.92	137.03	136.39	136.63
136.85	136.24	136.74	136.13	137.05	135.75	136.51
136.93	136.33	136.85	136.00	137.05	135.80	136.69
137.12	135.86	136.47	136.07	136.76	136.32	136.95
137.17	135.93	136.94	135.98	136.78	135.84	136.93

To simplify the calculations 136.00 is subtracted from all observations which are then multiplied by 100 as shown in Table 1b.

Table 1b

SIMPLIFIED ISOTOPIC RATIOS

	A_1	B_1	A_2	B_2	A_3	B_3	A_4
	77	4	101	18	47	-33	58
	104	-18	104	- 1	82	41	71
	92	23	40	-56	126	26	69
	99	- 9	58	- 8	103	39	63
	85	24	74	13	105	-25	51
	93	33	85	0	105	-20	69
	112	-14	47	7	76	32	95
	117	- 7	94	- 2	78	-16	93
T_1:	779	36	603	-29	722	44	569

The variance is unchanged by the subtraction or addition of a constant amount from every item.

The quantities necessary for the construction of the analysis of variance table are:

n, the number of observations in the i^{th} class;

p, the number of classes;

N, the total number of observations;

T_i, the sum of the n observations in the i^{th} class;

$\sum_i T_i^2/n$, the ratio of the sum of squares of class totals to the number of observations in the i^{th} class;

T, the grand total;

T^2/N, the correction for the mean;

$\sum_{i\gamma} x^2_{i\gamma}$, the sum of the squares of all observations.

The value of $\sum_{i\gamma} x^2_{i\gamma}$ is obtained most easily by summing the squares of the observations in each class since the sum of squares and the total of a given class can be obtained in one operation on most calculating machines.

Therefore, from the data, one tabulates

$n = 8$ $\qquad T^2/N = 132,503.1$

$p = 7$ $\qquad \sum_i T_i^2/n = 227,446.0$

$N = 56$ $\qquad \sum_{i\gamma} x^2_{i\gamma} = 252,222.0$

The variances are computed as

$$S = \sum_{i\gamma} x^2_{i\gamma} - T^2/N = 119,718.9$$

$$S_i = \sum_i T_i^2/n - T^2/N = 94,942.9$$

$$S_{\gamma(i)} = S - S_i = 24,776.0$$

Using these values the analysis of variance table is formed, Table 1c.

Table 1c

ANALYSIS OF VARIANCE TABLE

Source of Estimate	Sum of Squares	Degrees of Freedom	Mean Square
Between samples	$S_i = 94,942.9$	$p-1 = 6$	$S_i/(p-1) = 15,823.82$
Within samples	$S_{\gamma(i)} = 24,776.0$	$N-p = 49$	$S_{\gamma(1)}/(N-p) = 505.63$
Total	$S = 119,718.9$	$N-1 = 55$	

The F-test is now performed by calculating

$$F = \frac{S_i}{(p-1)} \Big/ \frac{S_{\gamma(i)}}{(N-p)} = 31.30$$

The calculated value for F is compared with the critical value for $F_{(p-1),\,(N-p),\,\alpha}$ obtained from tables of the percentage points of the F-distribution, i.e. $F_{6,\,49,\,0.05}$ for a significance test at the 5 per cent level. A value of F for the 49 degrees of freedom associated with the "within sample variation" is not listed. However, values for 40 and 60 degrees of freedom are given as 2.3359 and 2.2540 respectively. Since the calculated value, F = 31.30, is much greater than predicted, it is concluded that the sample and tank are significantly different.

The means are next compared by determining their standard deviations. The greatest comparison difference for the means is given by

$$s_{\bar{x}_i} \cdot W_{49,\ 0.05} = \sqrt{505.63/8} \cdot 4.19$$

$$= 33.31$$

where $s_{\bar{x}_i} = \sqrt{s^2/n}$ and $W_{49,\ 0.05}$ is obtained from tables of the studentized range for N-p degrees of freedom where $\alpha = 0.05$. In this test it is assumed that the sample variance is an adequate estimate of the standard deviation, σ.

The class means, T_i/n are arranged in order of increasing magnitude, i.e.,

B_2	B_1	B_3	A_4	A_2	A_3	A_1
-3.63	4.50	5.50	71.13	75.38	90.25	97.38

and the greatest comparison differences within and between the subgroups are compared with the calculated value, 33.31. The greatest differences within the subgroups are

$$B_2 - B_3 = 9.13$$
$$A_4 - A_1 = 26.25$$

while the least difference between the subgroups is

$$B_3 - A_4 = 65.63$$

Therefore it is concluded that all the sample means differ significantly from the tank means.

The data are next tested by the classification of means, a test which is more explicit than the comparison of means, especially in case of vagrant data. The greatest permissible difference between successive means of the same subgroup is given by

$$t_{49,\ 0.05} \cdot s_{\bar{x}_i} \cdot \sqrt{2} \quad = \quad 22.59$$

where $t_{49,\ 0.05}$ is obtained from tables of the percentage of the t distribution for N-p degrees of freedom and $\alpha = 0.05$. If the value of $t_{N-p,\alpha}$ is not given explicitly it can be calculated from adjacent values using the reciprocals of the degrees of freedom. The values of $t_{40,\ 0.05}$ and $t_{60,\ 0.05}$ are 2.0211 and 2.0003 respectively, hence $t_{49,\ 0.05} = 2.0096$.

The greatest differences between adjacent sample and tank means are

$$B_2 - B_1 \quad = \quad 8.13$$
$$A_2 - A_3 \quad = \quad 14.87$$

while the least difference between the subgroups is

$$B_3 - A_4 \quad = \quad 65.63$$

Again this indicates that the samples and tank are significantly different.

Since there are more than two means in each subgroup both the sample and tank observations are submitted to the following t-test.

For the sample means the grand mean $\bar{x}_m$ is equal to 2.13 and the greatest difference d_L between the sample means $\bar{x}_1$ and $\bar{x}_m$ is 5.75. Since this subgroup contains exactly three means the value of t is given by

$$t = \frac{\dfrac{d_L}{s_{\bar{x}_1}} - \dfrac{1}{2}}{\dfrac{1}{4} + \dfrac{1}{m}} = 0.28$$

where m is the number of degrees of freedom associated with the variance, i.e., N-p. The calculated value is less than the critical normal deviate, $t_{0.05} = 1.96$. It is concluded, therefore that the sample values form a single group.

For the standard means, $\bar{x}_m = 83.53$ and $d_L = 13.85$. Since there are more than three means in the subgroup t is computed as

$$t = \frac{\dfrac{d_L}{s_{\bar{x}_1}} - \dfrac{6}{5} \log_{10}{}^{k}}{3\left(\dfrac{1}{4} + \dfrac{1}{m}\right)} = 1.26$$

where k is the number of classes of the subgroup. Again the calculated value of t is less than 1.96 and it is concluded that the standard values form a single group.

The homogeneity of each group of three or more means is tested by forming an estimate of the variance from the means of each subgroup and comparing it with $s^2_{\bar{x}_i}$ by an F-test. The variance of a subgroup is obtained by dividing the sum of the squares of the differences between the grand mean $\bar{x}_m$ and the class means $\bar{x}_i$ by the number of degrees of freedom q-1 where q is the number of means in the subgroup.

For the three sample means the variance is given by

$$s_q^2 = \frac{\sum(\bar{x}_m - \bar{x}_i)^2}{q - 1} = \frac{50.09}{2} = 25.05$$

and $$F = \frac{63.20}{25.05} = 2.52$$

Since the calculated value of F is less than F_{49}, 2, 0.025, which lies between 39.473 and 39.481, the three sample means form an homogeneous group.

Similarly for the four tank means

$$s_q^2 = \frac{\sum(x_m - x_i)^2}{q - 1} = \frac{457.23}{3} = 152.41$$

and $$F = \frac{152.41}{63.20} = 2.41$$

This value calculated for F is less than F_{49}, 3, 0.025 which lies between 14.037 and 13.992. It is concluded that the standard means form an homogeneous group. To obtain a five per cent significance level in this test it is necessary to look up $F_{49,\ (q-1),\ 0.025}$ rather than $F_{49,\ (q-1),\ 0.05}$ since the larger number is always placed in the numerator.

Since the above tests indicate that there was no significant drift during the analysis, all the ratios in each subgroup are combined. For the tank classes the sum of all ratios, the number of observations and the mean are denoted by T_1, N_1 and $\overline{X}_1$, respectively, i.e.

$$T_1 = 2673$$

$$N_1 = 32$$

$$\overline{X}_1 = \frac{2673}{32} = 83.53$$

Similarly, for the sample classes the sum of all the ratios is denoted by T_2, the number of observations by N_2 and the mean by $\overline{X}_2$, i.e.,

$$T_2 = 51$$

$$N_2 = 24$$

$$\overline{X}_2 = \frac{51}{24} = 2.13$$

In addition to these quantities, the sum of squares $\sum_{ir} x^2_{ir}$ is needed to compute the pooled estimate of variance s_p^2 which is given by

$$s_p^{\,2} = \frac{\sum_{ir} x^2_{\;ir} - T_1^{\,2}/N_1 - T_2^{\,2}/N_2}{N_1 + N_2 - 2}$$

$$= \frac{252222 - (267_3)^2/32 - (51)^2/24}{32 + 24 - 2}$$

$$= 533.97389$$

and the pooled estimate of the standard deviation s_p is

$$s_p = 23.108$$

The quantity $N_1 + N_2 - 2$ defines the number of degrees of freedom associated with the pooled estimate of the variance.

A Student t-test is performed on the combined data. The value of t is

$$t = \frac{\overline{X}_1 - \overline{X}_2}{s_p\left[(1/N_1) + (1/N_2)\right]^{\frac{1}{2}}} = 13.05$$

Since this calculated value of t is much greater than $t_{54,\ 0.005}$, approximately 2.9, there is a significant difference between the tank and sample means at the one percent level.

A 95 percent confidence interval for the analysis is computed from the formula

$$\overline{X}_1 - \overline{X}_2 \pm t_{54,\ 0.025}\, s_p \sqrt{(1/N_1) + (1/N_2)}$$

The value of $t_{54,\ 0.025}$ must be calculated by interpolation of the reciprocals of the degrees of freedom for $t_{40,\ 0.025}$ and $t_{60,\ 0.025}$. The confidence interval is then

$$83.5 - 2.1 \pm 2.3057 \times 23.108 \times 0.27003$$

$$81.4 \pm 14.4$$

To obtain the confidence interval in terms of the original units the above result must be divided by 100, i.e.,

$$0.814 \pm 0.144$$

Similarly, the means may be converted to the original units by dividing by 100 and adding 136.00, i.e.,

$$\bar{X}_1 = 136.84$$

$$\bar{X}_2 = 136.02$$

The standard deviations may also be expressed in original units by dividing the computed values by 100.

Example 2: Sample B1 - 1

In this analysis it was necessary to reject one class of observations as a result of the comparison and classification of means. The calculations involved are summarized below. The reader is referred to Example 1 for a more detailed discussion.

The original data are tabulated in Tables 2a and 2b. In this example, 135.00 is subtracted from all observations and these values are multiplied by 100 to obtain whole numbers.

Table 2a

MASS SPECTROMETER DATA

A_1	B_1	A_2	B_2	A_3
135.38	134.83	135.62	135.00	135.83
135.43	134.50	135.86	135.05	135.62
135.57	134.86	135.81	135.02	135.84
135.55	135.00	135.73	135.06	135.92
135.73	135.27	135.83	134.87	135.96
135.64	134.81	135.41	134.95	135.78
135.42	135.24	135.60	134.78	135.84
135.68	134.22	135.91	134.83	135.88

Table 2b

SIMPLIFIED ISOTOPIC RATIOS

	A_1	B_1	A_2	B_2	A_3
	38	- 17	62	0	83
	43	- 50	86	5	62
	57	- 14	81	2	84
	55	0	73	6	92
	73	27	83	- 13	96
	64	- 19	41	- 5	78
	42	24	60	- 22	84
	68	- 78	91	- 17	88
T_i:	440	-127	577	- 44	667

The quantities required to construct the analysis of variance table are:

$n = 8$ $\qquad T = 1,513.0$

$p = 5$ $\qquad T^2/N = 57,229.2$

$N = 40$ $\qquad \Sigma_i T_i^2/n = 123,685.4$

$\Sigma_{i\gamma} x^2_{i\gamma} = 137,081.0$

$$S = \Sigma_{i\gamma} x^2_{i\gamma} - T^2/N = 79,851.8$$

$$S_i = \Sigma_i T_i^2/n - T^2/N = 66,456.2$$

$$S_{\gamma(i)} = S - S_i = 13,395.6$$

Table 2c

ANALYSIS OF VARIANCE TABLE

Source of Estimate	Sum of Squares	Degrees of Freedom	Mean Square
Between Samples	$S_1 = 66,456.2$	$p-1 = 4$	$S_i/(p-1) = 16,614.05$
Within Samples	$S_{\gamma(i)} = 13,395.6$	$N-p = 35$	$S_{\gamma(i)}/(N-p) = 382.73$
Total	$S = 79,851.8$	$N-1 = 39$	

$$F = \frac{16,614.05}{382.73} = 43.41$$

$F_{5,\ 39,\ 0.05}$ lies between 4.6233 and 4.3738, hence it is concluded that the tank and sample ratios are significantly different.

The estimate of the standard deviation $s_{\bar{x}_i}$ is equal to $\sqrt{382.73/8}$. The greatest comparison difference to be judged significant is

$$s_{\bar{x}_i} \cdot W_{4,\ 35,\ 0.05} = 6.917 \cdot 3.81$$
$$= 26.35$$

When the means are arranged in increasing order of magnitude,

B_1	B_2	A_1	A_2	A_3
- 15.88	- 5.50	55.00	72.13	83.38

it is found that the greatest differences within the subgroups are

$$A_1 - A_3 = 28.38$$
$$B_1 - B_2 = 10.38$$

while the least difference between the subgroups is

$$B_2 - A_1 = 60.50$$

Since the difference between $A_1 - A_3$ is greater than the value $s_{\bar{x}_i} \cdot W_{4,\ 35,\ 0.05}$ it is concluded that the means were not drawn from the same population.

In the classification of means the greatest difference between adjacent means is

$$t_{35,\ 0.05} \cdot s_{\bar{x}_i} \sqrt{2} = 2.0303 \cdot 6.917 \cdot 1.4142$$
$$= 19.86$$

The differences between the means of both the sample classes and tank classes are all less than this value while the least difference between the tank and sample means is greater than 19.86. Therefore, this test indicates that the two subgroups are significantly different.

The process terminates for the sample means as there are only two means in this subgroup. However, a t-test can be performed on the three tank means. For the tank means

$$\bar{x}_m = 70.17$$

$$d_L = 15.17$$

and

$$t = \frac{\left(\frac{d_L}{s_{\bar{x}_i}}\right) - \frac{1}{2}}{3\left(\frac{1}{4} + \frac{1}{35}\right)} = 2.03$$

which exceeds the normal critical deviate $t_{0.05} = 1.96$.

The means of the tank subgroup failed the comparison of means and the t-test, hence, it is concluded that this subgroup should be further divided. The grouping A_1 and A_2 - A_3 suggests itself since the difference between the means A_1 and A_2 is considerably larger than that between A_2 and A_3. Consequently, the observations in column A_1 are rejected and the data reevaluated for the remaining classes. For these classes

$n = 8$ $\qquad T = 1,073$

$p = 4$ $\qquad T^2/N = 35,979.0$

$N = 32$ $\qquad \sum_i T_i^2/n = 99,485.4$

$\sum_{i\gamma} x_{i\gamma}^2 = 111,701.0$

$$S = \sum_{i\gamma} x^2_{i\gamma} - T^2/N = 75,722.0$$

$$S_i = \sum_i T_i^2/n - T^2/N = 63,506.4$$

$$S_{\gamma(i)} = S - S_i = 12,215.6$$

Table 2c

ANALYSIS OF VARIANCE TABLE

Source of Estimate	Sum of Squares	Degrees of Freedom	Mean Square
Between Samples	$S_i = 63,506.4$	$p-1 = 3$	$S_i/(p-1) = 21,168.79$
Within Samples	$S_{\gamma(i)} = 12,215.6$	$N-p = 28$	$S_{\gamma(i)}/(N-p) = 436.27$
Total	$S = 75,722.0$	$N-1 = 31$	

$$F = \frac{21,168.79}{436.27} = 48.52$$

which is significantly greater than the value $F_{3,\ 28,\ 0.05} = 2.93$.

The estimate of the standard deviation $s_{\bar{x}_i}$ is equal to $\sqrt{436.27/8}$. The greatest comparison difference is

$$s_{\bar{x}_1} \cdot W_{3,\ 28,\ 0.05} = 7.385 \cdot 3.49$$
$$= 25.77$$

When the means are arranged in order of increasing magnitude,

B_1	B_2	A_2	A_3
-15.88	-5.50	72.13	82.38

it is seen that the differences between the means of both subgroups are less than the value of $s_{\bar{x}_1} \cdot W_{3,\ 28,\ 0.05}$ while the least difference between the subgroups is much greater than 25.77. The data now meet the comparison of means test.

In the classification of means test the difference that must be exceeded by successive means is given by

$$t_{28,\ 0.05}\, s_{\bar{x}_1} \sqrt{2} = 2.0484 \cdot 7.385 \cdot 1.4142$$
$$= 21.39$$

Therefore, the means separate into subgroups which are significantly different since the only difference exceeding 21.39 is that between the means B_2 and A_2. Since both subgroups now contain only two means the classification process terminates.

Designating the tank and sample ratios by the subscripts 1 and 2, respectively, compute

$$T_1 = 1244 \qquad T_2 = -171$$

$$N_1 = 16 \qquad N_2 = 16$$

$$\overline{X}_1 = 77.75 \qquad \overline{X}_2 = -10.69$$

$$s_p^2 = \frac{\sum_{i\gamma} x^2_{i\gamma} - T_1^2/N_1 - T_2^2/N_2}{N_1 + N_2 - 2}$$

$$= 438.4146$$

and $s_p = 20.938$

The value of t is computed as

$$t = \frac{\overline{X}_1 - \overline{X}_2}{s_p\sqrt{(1/N_1) + (1/N_2)}}$$

$$= 11.95$$

which is greater than $t_{30,\ 0.005} = 3.0298$ indicating that the tank and sample observations are significantly different at the one percent level.

Finally the 95 percent confidence interval is computed from the formula

$$\overline{X}_1 - \overline{X}_2 \pm t_{30,\ 0.025} \quad s_p\sqrt{\frac{1}{N_1} + \frac{1}{N_2}}$$

and converted into the original units by dividing by 100, i.e.,

$$0.884 \pm 0.175$$

Example 3: Intercomparison of Samples BB2-2 and B2-1

This example is included to illustrate the modified statistical treatments used for testing the intercomparisons. The mass spectrometer ratios are given in Tables 3a and 3b as before. To simplify the calculations 136.00 is subtracted from all observations which are then multiplied by 100. The 28/29 ratios of samples BB2-2 and B2-1 are tabulated in columns A and B, respectively, of Tables 3a and 3b.

Table 3a

MASS SPECTROMETER DATA

A_1	B_1	A_2	B_2	A_3	B_3	A_4	B_4
136.61	136.02	135.88	135.89	136.09	135.81	136.23	135.42
136.20	135.66	136.42	135.95	136.13	135.79	135.96	135.63
136.45	135.92	136.05	135.76	136.38	135.87	136.39	135.71
136.18	135.97	136.13	136.34	136.10	135.99	135.92	135.66
136.01	135.96	136.36	135.96	136.49	135.73	136.14	135.92
136.41	135.74	136.49	135.79	135.60	135.90	136.09	135.97
136.09	136.12	136.05	136.15	135.89	136.14	136.08	136.06
136.39	135.92	136.21	135.82	136.03	135.82	136.13	135.75

Table 3b

SIMPLIFIED ISOTOPIC RATIOS

	A_1	B_1	A_2	B_2	A_3	B_3	A_4	B_4
	61	2	-12	-11	9	-19	23	-58
	20	-34	42	- 5	13	-21	- 4	-37
	45	- 8	5	-24	38	-13	39	-29
	18	- 3	13	34	10	- 1	- 8	-34
	1	- 4	36	- 4	49	-27	14	- 8
	41	-26	49	-21	-40	-10	9	- 3
	9	12	5	15	-11	14	8	6
	39	- 8	21	-18	3	-18	13	-25
T_i:	234	-69	159	-34	71	-95	94	-188

The quantities required to construct the analysis of variance table are:

$n = 8$

$p = 8$

$N = 64$

$T = 172$

$T^2/N = 462.3$

$\sum_i T_i^2/n = 18{,}025.0$

$\sum_{i\gamma} x^2_{i\gamma} = 39{,}386.0$

$$S = \sum_{i\gamma} x^2_{i\gamma} - T^2/N = 38{,}923.7$$

$$S_i = \sum_i T_i^2/n - T^2/N = 17{,}562.7$$

$$S_{\gamma(i)} = S - S_i = 21{,}361.0$$

Table 3c

ANALYSIS OF VARIANCE TABLE

Source of Estimate	Sum of Squares	Degrees of Freedom	Mean Square
Between Samples	$S_i = 17{,}562.7$	$p-1 = 7$	$S_i/(p-1) = 2{,}508.96$
Within Samples	$S_{\gamma(i)} = 21{,}361.0$	$N-p = 56$	$S_{\gamma(i)}/(N-p) = 381.44$
Total	$S = 38{,}923.7$	$N-1 = 63$	

$$F = \frac{2{,}508.96}{381.44} = 6.58$$

Since this value of F is greater than $F_{7,\ 56,\ 0.05} = 2.18$ it is concluded that the two samples differ significantly and that further examination is warranted.

The estimate of the standard deviation $s_{\bar{x}_i}$ is equal to $\sqrt{381.44/8}$ and the greatest comparison difference that is to be judged significant is

$$s_{\bar{x}_i} \cdot W_{7,\ 56,\ 0.05} = 6.905 \cdot 4.32$$
$$= 29.83$$

When the class means are arranged in order of increasing magnitude, i.e.,

B_4	B_3	B_1	B_2	A_3	A_4	A_2	A_1
-23.50	-11.88	-8.63	-4.25	8.88	11.75	19.88	29.25

it is found that the greatest differences within the class means are

$$B_4 - B_2 = 19.25$$

$$A_3 - A_1 = 20.37$$

which are less than 29.83. The least difference between the class means

$$B_2 - A_3 = 13.13$$

is less than 29.83, a result which is not surprising in view of the small isotopic differences between the two samples.

The difference between adjacent means which is to be judged significant is

$$t_{56,\ 0.05} \cdot s_{\bar{x}_1} \sqrt{2} = 2.0033 \cdot 6.905 \cdot 1.4142$$
$$= 19.55$$

The greatest differences between adjacent means of the two subgroups are

$$B_4 - B_3 = 11.62$$

$$A_2 - A_1 = 9.37$$

In both subgroups the differences are less than 19.55. The least difference between the means of the subgroups, $B_2 - A_3 = 13.13$, is less than the calculated value 19.55 indicating some overlap.

For the B classes:

$$\bar{x}_m = -12.07$$

$$d_L = 11.43$$

and $$t = \frac{\frac{d_L}{s_{\bar{x}_i}} - \frac{6}{5}\log_{10}4}{3\left(\frac{1}{4} + \frac{1}{56}\right)} = 1.16$$

Similarly for the A classes

$$\bar{x}_m = 17.44$$

$$d_L = 11.81$$

and $$t = \frac{\frac{d_L}{s_{\bar{x}_i}} - \frac{6}{5}\log_{10}4}{3\left(\frac{1}{4} + \frac{1}{56}\right)} = 1.23$$

Since both of these values are less than the critical normal deviate $t_{0.05} = 1.96$ it is concluded that the two samples form single groups.

The homogeneity of each groups is next tested. For the A class means

$$s_q^2 = \frac{\sum(\bar{x}_i - \bar{x}_m)^2}{4 - 1} = 83.69$$

and $$F = \frac{s_q^2}{s_{\bar{x}_i}^2} = \frac{83.69}{47.68}$$

$$= 1.76$$

Similarly for the B class means

$$s_q^2 = \frac{\sum(\overline{x}_i - \overline{x}_m)^2}{4 - 1} = 67.89$$

and $$F = \frac{67.89}{47.68} = 1.42$$

Since the calculated value of F for both subgroups is less than $F_{56,\ 4,\ 0.025}$, which lies between 14.037 and 13.992, it is concluded that the two samples come from homogeneous populations.

Designating the A and B classes by the subscripts 1 and 2 respectively compute:

$$\begin{array}{lcl@{\qquad\qquad}lcl} T_1 & = & 558 & T_2 & = & -386 \\ N_1 & = & 32 & N_2 & = & 32 \\ \overline{X}_1 & = & 17.44 & \overline{X}_2 & = & -12.06 \end{array}$$

$$s_p^2 = \frac{\sum_{i\gamma} x^2_{i\gamma} - T_1^2/N_1 - T_2^2/N_2}{N_1 + N_2 - 2}$$

$$= 403.2218$$

and $$s_p = 20.080$$

The combined data are examined by the Student t-test, i.e.

$$t = \frac{\overline{X}_1 - \overline{X}_2}{s_p \sqrt{(1/N_1) + (1/N_2)}} = 5.88$$

which is greater than $t_{62,\ 0.005} = 2.9111$. Therefore, it is concluded that the two samples are significantly different at the one per cent level.

The 95 per cent confidence interval is computed from the formula:

$$\bar{X}_1 - \bar{X}_2 \pm t_{62,\ 0.025} \quad s_p \sqrt{(1/N_1) + (1/N_2)}$$

$$29.5 \pm 11.5$$

The confidence interval is converted to the original units by dividing by 100, i.e.,

$$0.180 \pm 0.115$$

At this point it is convenient to review the statistical procedure adopted for the intercomparisons. Analyses, for which confidence intervals were computed, successfully passed the F-test and the Student t-test for combined ratios. However, in most instances the least difference between the means of the two subgroups was less than the values computed for the greatest comparison difference and the difference between adjacent means. In such instances reliance was placed on the significance of the F-test and the Student t-test. The tests outlined for the comparison of means and the classification of means were used only to check for drift during the analysis. Any classes which fell outside the calculated limits were rejected.

Example 4: Intercomparison of Samples BB2-3 and BC2-4

This intercomparison is included to illustrate the case where there was no significant difference between the samples. The 28/29 ratios are tabulated in Tables 4a and 4b. In this example 135.00 is subtracted from all observations and these values are multiplied by 100.

Table 4a

MASS SPECTROMETER DATA

A_1	B_1	A_2	B_2	A_3	B_3	A_4	B_4	A_5
135.81	136.07	136.12	135.86	135.89	135.94	135.24	135.49	135.62
136.31	135.86	135.82	136.45	135.59	136.03	135.90	135.61	135.40
135.58	135.84	135.56	136.22	135.84	135.57	135.66	136.04	135.71
135.68	135.61	135.51	135.89	136.23	135.79	135.42	136.19	135.84
135.42	136.00	135.37	135.61	135.81	136.09	135.86	136.16	135.65
135.94	135.50	135.97	135.82	135.34	135.96	135.65	135.85	136.09
135.26	135.65	135.45	136.16	135.96	135.79	135.51	135.96	135.57
135.51	135.86	135.43	135.42	135.64	136.00	135.92	135.99	136.12

Table 4b

SIMPLIFIED ISOTOPIC RATIOS

	A_1	B_1	A_2	B_2	A_3	B_3	A_4	B_4	A_5
	81	107	112	86	89	94	24	49	62
	131	86	82	145	59	103	90	61	40
	58	84	56	122	84	57	66	104	71
	68	61	51	89	123	79	42	119	84
	42	100	37	61	81	109	86	116	65
	94	50	97	82	84	96	65	85	109
	26	65	45	116	96	79	51	96	57
	51	86	43	42	64	100	92	99	112
T_i:	551	639	523	743	680	717	516	729	600

The quantities required to construct the analysis of variance table are:

$n = 8$

$p = 9$

$N = 72$

$T = 5{,}698$

$T^2/N = 450{,}933.3$

$\sum_i T_i^2/n = 458{,}960.7$

$\sum_{i\gamma} x_{i\gamma}^2 = 500{,}386.0$

$S = \sum_{i\gamma} x_{i\gamma}^2 - T^2/N = 49{,}452.7$

$S_i = \sum_i T_i^2/n - T^2/N = 8{,}027.4$

$S_{\gamma(i)} = S - S_i = 41{,}425.3$

Table 4c

ANALYSIS OF VARIANCE TABLE

Source of Estimate	Sum of Squares	Degrees of Freedom	Mean Square
Between Samples	$S_i = 8{,}027.4$	$p-1 = 8$	$S_i/(p-1) = 1{,}003.43$
Within Samples	$S_{\gamma(i)} = 41{,}425.3$	$N-p = 63$	$S_{\gamma(i)}/(N-p) = 657.54$
Total	$S = 49{,}452.7$	$N-1 = 71$	

$$F = \frac{1{,}003.43}{657.54} = 1.526$$

Since the calculated value of F is less than $F_{8,\ 63,\ 0.05} = 2.089$ it is concluded that there is no significant difference between the two samples. This is verified by the Student t-test for the combined data:

$$t = \frac{\overline{X}_1 - \overline{X}_2}{s_p\sqrt{(1/N_1) + (1/N_2)}} = 2.780$$

which is less than $t_{70,\ 0.005} = 2.8990$.

Results such as these indicate that there is no isotopic difference between the samples, i.e., a zero isotope effect.

APPENDIX II

Derivation of Confidence Intervals for Combined Analyses

The confidence intervals computed for individual samples prepared under the same experimental conditions or repeated analyses of the same sample can be combined and expressed as a single value, the confidence interval for combined analyses. The derivation of the equation for the computation of the confidence interval for combined analyses is outlined in the following sections.

For r analyses there are r pairs of means $\bar{X}_{i_1}$, $\bar{X}_{i_2}$, where $\bar{X}_{i_1}$ is the mean of N_{i_1} observations from a normal population with mean μ_1 and variance σ^2. $\bar{X}_{i_2}$ is the mean of N_{i_2} observations from a normal population with mean μ_2 and variance σ^2 and $i = 1, \ldots, r$.

Define a function

$$Z_i = \bar{X}_{i_1} - \bar{X}_{i_2} \qquad (85)$$

which has a normal distribution with a mean $\mu_1 - \mu_2$ and variance

$$\sigma^2 Z_i = \sigma^2\left(\frac{1}{N_{i_1}} + \frac{1}{N_{i_2}}\right) = \sigma_i^2. \qquad (86)$$

Further define the functions

$$E(Z_i) = \mu_1 - \mu_2 \qquad (115)$$

and $$Y = \sum_{i=1}^{r} \ell_i Z_i \qquad (116)$$

Then $$E(Y) = (\mu_1 - \mu_2) \qquad (117)$$

and

$$\sigma_Y^2 = \sum_{i=1}^{r} \ell_i \, \sigma_i^2 \qquad (118)$$

The quantities $E(Y)$ and $\sigma^2{}_Y$ represent the true mean and variance when $\sum_{i=1}^{r} \ell_i = 1$. Therefore select the value of the ℓ_i's to obtain a minimum value of $\sigma^2{}_Y$. Let

$$A = \sum_{i=1}^{r} \ell_i^2 \sigma^2 - \lambda(\sum \ell_i - 1) \qquad (119)$$

Differentiation of equation (119) gives:

$$\frac{\partial A}{\partial \ell_i} = 2\sum_{i=1}^{r} {}_i\sigma^2 - \lambda = 0 \qquad (120)$$

and $$\sum_{i=1}^{r} \ell_i = \frac{\lambda}{2} \; \frac{1}{\sum_{i=1}^{r} \sigma_i^2} \qquad (121)$$

Since $$\sum_{i=1}^{r} \ell_i = 1$$

$$\frac{\lambda}{2} = \frac{1}{\sum_{i=1}^{r} 1/\sigma_i^2} \qquad (122)$$

and $$\ell_i = \frac{1/\sigma_i^2}{\sum_{i=1}^{r} 1/\sigma_i} . \qquad (123)$$

Equation (86) is written as

$$\frac{1}{\sigma_i^2} = \frac{1}{\sigma^2(1/N_{i_1} + 1/N_{i_2})} \qquad (124)$$

$$= \frac{N_{i_1} \; N_{i_2}}{\sigma^2(N_{i_1} + N_{i_2})} \qquad (125)$$

$$= \frac{b_i}{\sigma^2} \qquad (126)$$

where $$b_i = \frac{N_{i_1} \; N_{i_2}}{N_{i_1} + N_{i_2}} . \qquad (88)$$

Therefore $$\ell_i = \frac{b_i}{\sum_{i=1}^{r} b_i} . \qquad (127)$$

Now $$\sigma_y^2 = \sum_{i=1}^{r} \ell^2{}_i \, \sigma_i^2$$

$$= \sum_{i=1}^{r} \frac{b^2{}_i}{(\sum_{i=1}^{r} b_i)^2} \; \frac{\sigma^2}{b_i}$$

$$= \frac{\sigma^2}{\sum_{i=1}^{r} b_i} . \qquad (128)$$

Since $$f(Z_i) = N(\mu_1 - \mu_2; \sigma_i^2) \qquad (129)$$

$$f(Y) = N(\mu_1 - \mu_2; \frac{\sigma^2}{\sum_{i=1}^{r} b_i}). \qquad (130)$$

The pooled estimate of the variance is given by

$$s_p^2 = \frac{\sum_{i\gamma} x_{i\gamma}^2 - \sum_{i=1} T_{i_1}^2/N_{i_1} - \sum_{i=1} T_{i_2}^2/N_{i_2}}{\sum_{i=1}^{r} (N_{i_1} + N_{i_2}) - 2r} \qquad (90)$$

where $\sum_{i\gamma} x_{i\gamma}^2$ is the sum of the squares of all observations, T_{i_1} is the sum of the observations made on the first variable in the i^{th} experiment and T_{i_2} is the sum of the observations made on the second variable in the i^{th} experiment.

The 95 per cent confidence interval for $\mu_1 - \mu_2$ is given by the formula

$$Y \pm t_{k,\, 0.025}\, s_p \frac{1}{\sqrt{\sum_{i=1}^{r} b_i}} \qquad (92)$$

where $$k = \sum_{i=1}^{r} (N_{i_1} - N_{i_2}) - 2r \qquad (91)$$

that is, the total number of observations minus twice the number of experiments.

In the special case where $N_{i_1} - N_{i_2} = N$, the 95 per cent confidence interval for $\mu_1 - \mu_2$ is

$$Y \pm t_{k,\ 0.025}\, s_p \sqrt{2/Nr} \qquad (92)$$

where $k = 2r(N-1)$.

Finally if $r = 1$, the confidence interval reduces to

$$Y \pm t_{k,\ 0.025}\, s_p \sqrt{2/N} \qquad (131)$$

where $k = 2(N-1)$.

Computation of Confidence Intervals for Combined Analyses

The confidence interval for combined analyses can be computed most conveniently in the following manner. The i^{th} experiment consists of N_{i_1} observations from the first population and N_{i_2} observations from the second population, i.e., tank and sample nitrogen, respectively. The quantities b_i and ℓ_i are computed by arranging the data as shown in Table 1.

Table 1

COMPUTATION OF b_i AND ℓ_i

N_{i_1}	N_{i_2}	$(N_{i_1})(N_{i_2})$	$(N_{i_1} + N_{i_2})$	$b = \dfrac{(N_{i_1})(N_{i_2})}{(N_{i_1} + N_{i_2})}$	$\ell_i = \dfrac{b_i}{\sum_{i=1}^{r} b_i}$
N_{1_1}	N_{1_2}	N_{1_1} N_{1_2}	$N_{1_1} + N_{1_2}$	b_1	ℓ_1
N_{2_1}	N_{2_2}	N_{2_1} N_{2_2}	$N_{2_1} + N_{2_2}$	b_2	ℓ_2
N_{3_1}	N_{3_2}	N_{3_1} N_{3_2}	$N_{3_1} + N_{3_2}$	b_3	ℓ_3
$\vdots$	$\vdots$	$\vdots$ $\vdots$	$\vdots$	$\vdots$	$\vdots$
N_{r_1}	N_{r_2}	N_{r_1} N_{r_2}	$N_{r_1} + N_{r_2}$	b_r	ℓ_r
			$\sum_{i=1}^{r}(N_{r_1} + N_{r_2})$	$\sum_{i=1}^{r} b_i$	1

The sum of the observations in the first population is denoted by T_{i_1} and the mean is given by

$$\bar{X}_{i_1} = T_{i_1}/N_{i_1}. \qquad (79)$$

Similarly for the second population the sum of the observations is T_{i_2} and the mean is

$$\bar{X}_{i_2} = T_{i_2}/N_{i_2}. \qquad (81)$$

The value of $Y = \sum_{i=1}^{r} {}_iZ_i$ for the combined experiments is obtained as shown in Table 2. It will be noted that the contribution that any experiment makes to the value of Y is governed by the number of observations in that experiment.

Table 2

COMPUTATION OF Y FOR COMBINED EXPERIMENTS

$\bar{X}_{i_1}$	$\bar{X}_{i_2}$	$Z_i = \bar{X}_{i_1} - \bar{X}_{i_2}$	$\ell_i Z_i$
$\bar{X}_{1_1}$	$\bar{X}_{1_2}$	$Z_1 = \bar{X}_{1_1} - \bar{X}_{1_2}$	$\ell_1 Z_1$
$\bar{X}_{2_1}$	$\bar{X}_{2_2}$	$Z_2 = \bar{X}_{2_1} - \bar{X}_{2_2}$	$\ell_2 Z_2$
$\bar{X}_{3_1}$	$\bar{X}_{3_2}$	$Z_3 = \bar{X}_{3_1} - \bar{X}_{3_2}$	$\ell_3 Z_3$
$\vdots$	$\vdots$	$\vdots$	$\vdots$
X_{r_1}	X_{r_2}	$Z_r = \bar{X}_{r_1} - X_{r_2}$	$\ell_r Z_r$
			Y

The pooled estimate of the standard deviation s_p^2 is given by

$$s_p^2 = \frac{\sum_{i\gamma} x_{i\gamma}^2 - \sum_{i=1}^{r} T_{i_1}^2/N_{i_1} - \sum_{i=1}^{r} T_{i_2}^2/N_{i_2}}{k} \tag{90}$$

where $\sum_{i\gamma} x_{i\gamma}^2$ is the sum of the squares of all observations and

$$k = \sum_{i=1}^{r} (N_{i_1} + N_{i_2}) - 2r \, . \tag{91}$$

After s_p is computed, the 95 per cent confidence interval for $\mu_1 - \mu_2$ is obtained from the formula

$$Y \pm t_{k,\, 0.025}\, s_p \frac{1}{\sqrt{\sum_{i=1}^{r} b_i}} \tag{92}$$

REFERENCES

(1) Stevenson, D.P., Wagner, C.D., Beeck, O. and Otvos, J.W., J. Chem. Phys., 16, 993-4 (1948).

(2) Beeck, O., Otvos, J.W., Stevenson, D.P. and Wagner, C.D., J. Chem. Phys., 16, 255-6 (1948).

(3) Yankwich, P.E. and Calvin, M., J. Chem. Phys., 17, 109-10 (1949).

(4) Bigeleisen, J. and Friedman, L., J. Chem. Phys., 17, 998-9 (1949).

(5) Lindsay, J.G., McElcheran, D.E. and Thode, H.G., J. Chem. Phys., 17, 589 (1949).

(6) Bigeleisen, J., J. Chem. Phys., 17, 675-8 (1949).

(7) Bigeleisen, J. and Mayer, M.G., J. Chem. Phys., 15, 261-7 (1947).

(8) Sheppard, W.A., Bader, R.F.W. and Bourns, A.N., Can. J. Chem., 32, 345-50 (1954).

(9) Glasstone, S., A chapter in "Theoretical Chemistry", D. Van Nostrand Co., Inc., New York, (1950).

(10) Eyring, H., J. Chem. Phys., 3, 107-15 (1935).

(11) Slater, N.B., Proc. Roy. Soc., A194, 112-31 (1948).

(12) Bigeleisen, J. and Wolfsberg, M., J. Chem. Phys., 21, 1972-4 (1953).

(13) Bigeleisen, J. and Wolfsberg, M., J. Chem. Phys., 22, 1264 (1954).

(14) Bigeleisen, J., J. Phys. Chem., 56, 823-8 (1952).

(15) Lindsay, J.G., Bourns, A.N. and Thode, H.G., Can. J. Chem., 29, 192 (1951).

(16) Bernstein, R.B., J. Phys. Chem., 56, 893-6 (1952).

(17) Yankwich, P.E., Stivers, E.C. and Nystrom, R.F., J. Chem. Phys., 20, 344 (1952).

(18) Yankwich, P.E. and Stivers, E.C., J. Chem. Phys., 21, 61 (1953).

(19) Yankwich, P.E. and Promislow, A.L., J. Am. Chem. Soc., 76, 4648-51 (1954).

(20) Yankwich, P.E., Promislow, A.L. and Nystrom, R.F., J. Am. Chem. Soc., 76, 5893-4 (1954).

(21) Yankwich, P.E. and Belford, R.L., J. Am. Chem. Soc., 76, 3067-70 (1954).

(22) Yankwich, P.E. and Weber, H.S., J. Am. Chem. Soc., 78, 564-7 (1956).

(23) Friedman, L. and Bigeleisen, J., J. Chem. Phys., 18, 1325-30 (1950).

(24) Stacey, F.W., Lindsay, J.G. and Bourns, A.N., Can. J. Chem., 30, 135-45 (1952).

(25) Bigeleisen, J., Can. J. Chem., 30, 443-7 (1952).

(26) Roe, A. and Hellman, M., J. Chem. Phys., 19, 660 (1951).

(27) Fry, A. and Calvin, M., J. Phys. Chem., 56, 897-901 (1952).

(28) Fry, A. and Calvin, M., J. Phys. Chem., 56, 901-5 (1952).

(29) Ropp, G.A., J. Chem. Phys., 23, 2196 (1955).

(30) Stevens, W.H., Pepper, J.M. and Lounsbury, M., J. Chem. Phys., 20, 192 (1952).

(31) Pitzer, K.S. and Gelles, E., J. Am. Chem. Soc., 75, 5132-3 (1953).

(32) Gelles, E. and Pitzer, K.S., J. Am. Chem. Soc., 77, 1974-8 (1955).

(33) Gelles, E. and Reid, R.I., Nature, 176, 1262 (1955).

(34) Gelles, E. and Clayton, J.P., Trans. Far. Soc., 52, 353-7 (1956).

(35) Lindsay, J.G., Bourns, A.N. and Thode, H.G., Can. J. Chem., 30, 163-5 (1952).

(36) Yankwich, P.E., Belford, R.L. and Fraenkel, G., J. Am. Chem. Soc., 75, 832-5 (1953).

(37) Yankwich, P.E. and Belford, R.L., J. Am. Chem. Soc., 75, 4178-82 (1953).

(38) Yankwich, P.E. and Webber, H.S., J. Am. Chem. Soc., 77, 4513-6 (1955).

(39) Bothner-By, A.A. and Bigeleisen, J., J. Chem. Phys., 19, 755-9 (1951).

(40) Bigeleisen, J. and Allen, T.L., J. Chem. Phys., 19, 760-4 (1951).

(41) Stevens, W.H. and Crowder, D.A., Can. J. Chem., 32, 792-801 (1954).

(42) Carrick, W.L. and Fry, A., J. Am. Chem. Soc., 77, 4381-7 (1955).

(43) Menschutkin, N., Z. physikal Chem., 6, 41-57, (1890).

(44) Laidler, K.J. and Hinshelwood, C.N., J. Chem. Soc., 1938, 858-62.

(45) Winkler, C.A. and Hinshelwood, C.N., J. Chem. Soc., 1935, 1147-51.

(46) Raines, H.C. and Hinshelwood, C.N., J. Chem. Soc., 1939, 1378-83.

(47) Grimm, H.G., Ruff, H. and Wolff, H., Z. physikal Chem., B13, 301-15 (1931).

(48) Stevens, W.H., Ph.D. Dissertation, McMaster University, 1952.

(49) Rittenberg, D., a chapter in "Preparation and Measurement of Isotopic Tracers". J.W. Edwards Co., Ann Arbor, Mich. (1946).

(50) Holt, P.F. and Hughes, B.P., J. Chem. Soc., 1953, 1666-9.

(51) Holt, P.F. and Hughes, B.P., J. Chem. Soc., 1955, 98-100.

(52) Ory, R.L., Prescott, J.M. and Lyman, C.M., J. Am. Chem. Soc., 76, 1449-50 (1954).

(53) Jones, S.L. and Trenner, N.R., Anal. Chem., 28, 387-90 (1956).

(54) Willits, C.O., Coe, M.R. and Ogg, C.L., J. Assoc. Offic. Agr. Chem., 32, 118-27 (1949).

(55) Willits, C.O. and Ogg, C.L., J. Assoc. Offic. Agr. Chem., 33, 179-88 (1950).

(56) Crichton, D.C., J. Chem. Soc., 91, 1793-7 (1907).

(57) Stewart, T.D. and Aston, J.G., J. Am. Chem. Soc., 49, 1718-28 (1927).

(58) Thode, H.G., Graham, R.L., and Ziegler, J.A., Can. J. Res., 23B, 40-7 (1945).

(59) Vogel, A.I., "A Textbook of Practical Organic Chemistry", Longmans, Green and Co., London, (1948).

(60) Vogel, A.I., "A Textbook of Practical Organic Chemistry", Longmans, Green and Co., London, (1948).

(61) Sprinson, D.B. and Rittenberg, D., J. Biol. Chem., 180, 707-14 (1949).

(62) Heilskov, N.S.C., Olesen, K., Schønheyder, F. and Werner, S., Scand. J. Clin. and Lab. Invest., 5, 366-75 (1953).

(63) Riley, R.F., Richter, E., Rotheram, M., Todd, N., Myers, L. and Nusbaum, R., J. Am. Chem. Soc., 76, 3301-3 (1954).

(64) Bennett, C.A. and Franklin, N.L., A chapter in "Statistical Analysis in Chemistry and the Chemical Industry", John Wiley and Sons, Inc., New York, (1954).

(65) Saunders, W.H. and Ašperger, S., J. Am. Chem. Soc., 79, 1612-5 (1957).

(66) Young, C.W., Koehler, J.S. and McKinney, D.S., J. Am. Chem. Soc., 69, 1410-15 (1947).

(67) Tomlinson, R., Private Communication.

(68) Bigeleisen, J. and Wolfsberg, M., "Theoretical and Experimental Aspects of Isotope Effects in Chemical Kinetics", a chapter in "Advances in Chemical Physics", Interscience Publishers, Inc., New York, (To be Published).

(69) Brown, H.C. and Taylor, M.D., J. Am. Chem. Soc., 69, 1332-6 (1947).