[CONTRIBUTION FROM THE CHEMISTRY DIVISION OF THE OAK RIDGE NATIONAL LABORATORY]

The Determination of the Isotope Effect and its Variation with Temperature in the Dehydration of Formic-C^{14} Acid[1,2]

BY GUS A. ROPP, A. J. WEINBERGER AND O. KENTON NEVILLE

An isotope effect of 11.11 ± 0.52% at 0° and of 8.59 ± 0.58% at 24.75° has been measured in the dehydration of formic-C^{14} acid in sulfuric acid. These values, expressed as $100(k_{12} - k_{14})/k_{12}$, correspond to a difference of 189 ± 53 calories per mole between the Arrhenius activation energies for the reaction of formic-C^{12} acid and formic-C^{14} acid. A sensitive flow method of measurement has been developed, which is applicable in the study of first-order reactions yielding a gaseous radioactive product.

Isotope effects which have been recently reported[3] in the reactions of isotopic carbon-labeled organic compounds are of importance because of the possibility that such effects can cast doubt on the conclusions drawn from tracer experiments in chemical and biological systems. In the present work the effect of temperature on the magnitude of a simple isotope effect is reported.

Tracer-labeled formic-C^{14} acid was dehydrated in excess 95% sulfuric acid at four temperatures. At each temperature the evolved carbon monoxide was passed through a small ion chamber where its instantaneous specific activity was measured and automatically plotted as a function of time by a Brown Recorder used with a Vibrating Reed Electrometer. In each case the first-order reaction rate constant, k_{12}, was determined simultaneously by measurement of the change of the total gas volume with time.

At each temperature the difference, $k_{12} - k_{14}$, in the reaction rate constants for the two species was determined from the slope of the linear plot of the logarithm of the specific activity against time and the isotope effect was calculated as $100(k_{12} - k_{14})/k_{12}$.

Experimental

A. Preparation of Reagents. (1) **Formic-C^{14} Acid Azeotrope.**—Labeled formic acid was prepared by dissolving dry C^{14}-labeled sodium formate in the non-radioactive formic acid–water azeotrope and distilling the radioactive azeotrope from the solution through a 40-inch Vigreux column. The distillate was a clear, water-white liquid, b.p. 106° at 736 mm. containing 77% formic acid. The average specific activity of formic acid used in determining the isotope effect was 0.30 μc. per mmole.

(2) **Sulfuric Acid.**—C.P. sulfuric acid was degassed by stirring 20 hours at room temperature at a pressure of less than 1 mm. Analysis showed that it contained 94.8% sulfuric acid.

B. Flow Method.—In the flow method of evaluating the isotope effect, the apparatus shown in Fig. 1 was used. A 2.36-g. portion of labeled formic acid azeotrope was added to 234 g. of sulfuric acid in a Pyrex vessel (A) in a thermostated bath. The solution was stirred continually by means of a magnetic bar. The formic acid was washed in with 30 g. of sulfuric acid which had been previously cooled sufficiently to prevent the temperature of the reaction mixture from rising above the bath temperature as a result of the addition of the formic acid. The evolved carbon monoxide was passed through a copper heat exchanger (B) to ensure equilibration with the temperature of the room and the ion chamber, and through an Ascarite–Drierite trap (C) to remove entrained water, formic acid or sulfuric acid. The pressure of the gas was indicated by a mercury manometer (F). As the carbon monoxide entered the 10-ml. stainless steel ion chamber (D), its instantaneous radioactivity per chamberful was measured continuously. After the gas passed through the Pauling oxygen meter, it was collected over dibutyl phthalate (E). The volume was recorded as a function of time and corrected to standard conditions for the calculation of k_{12}.

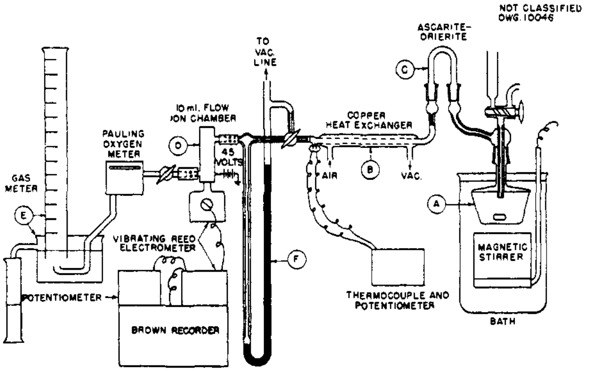

Fig. 1.—Isotope effect in formic acid dehydration: apparatus used in continuous measurement of gas volume and specific activity of carbon monoxide evolved.

The ion current generated in chamber D passed through a 10^{12}-ohm resistor. The potential developed across the resistor was amplified by the Vibrating Reed Electrometer and recorded continuously by the Brown Recorder. A potentiometer was introduced into the circuit in order to balance out most of the potential. The change in recorded potential (specific activity) during the run due to the isotope effect could then be plotted on about one full scale of the Brown Recorder.

Through the Brown Recorder plot of millivolts developed *vs.* time, a smooth curve was drawn. For each point taken from this curve, its millivolts reading was added to the potentiometer reading in millivolts to give the activity per chamberful (expressed as millivolts potential developed across the 10^{12}-ohm resistor) at the time, t. This total number of millivolts was then corrected to a standard temperature and pressure by use of an empirically determined factor which corrected for changes in moles of gas in the chamber as well as for the dependence of chamber efficiency on the pressure. For a number of such corrected values of the activity (expressed as millivolts potential developed per chamberful) the logarithms of the values were plotted against time, t, and $k_{12} - k_{14}$ was calculated as 2.303 times the slope. Figure 2 shows two such plots for experiments at 0° and the corresponding reaction rate curves. The "free volume" referred to is the air space in the apparatus. For the purpose of these plots, only values of t between 30 and 70% reaction were considered for two reasons: (a) kinetic studies showed that the reaction was purely first-order with respect to for-

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(2) Presented in part at the 119th National Meeting of the American Chemical Society, Cleveland, Ohio, April, 1951.

(3) O. Beeck, J. Otvos, D. Stevenson and C. Wagner, *J. Chem. Phys.*, **16**, 255 (1948); D. Stevenson, C. Wagner, O. Beeck and J. Otvos, *ibid.*, **16**, 993 (1948); F. Daniels and A. Meyerson, *Science*, **108**, 676 (1948); P. Yankwich and M. Calvin, *J. Chem. Phys.*, **17**, 109 (1949); J. Bigeleisen, *ibid.*, **17**, 425 (1949); W. Stevens and R. Attre, *Can. J. Research*, **B27**, 807 (1949); K. Pitzer, *J. Chem. Phys.*, **17**, 1341 (1949); W. Stevens and R. Attre, *ibid.*, **18**, 574 (1950); J. G. Lindsay, D. E. McElcheran and H. G. Thode, *ibid.*, **17**, 589 (1949); J. G. Lindsay, A. N. Bourns and H. G. Thode, *Can. J. Chem.*, **29**, 192 (1951); J. Bigeleisen and L. Friedman, *J. Chem. Phys.*, **17**, 998 (1949).

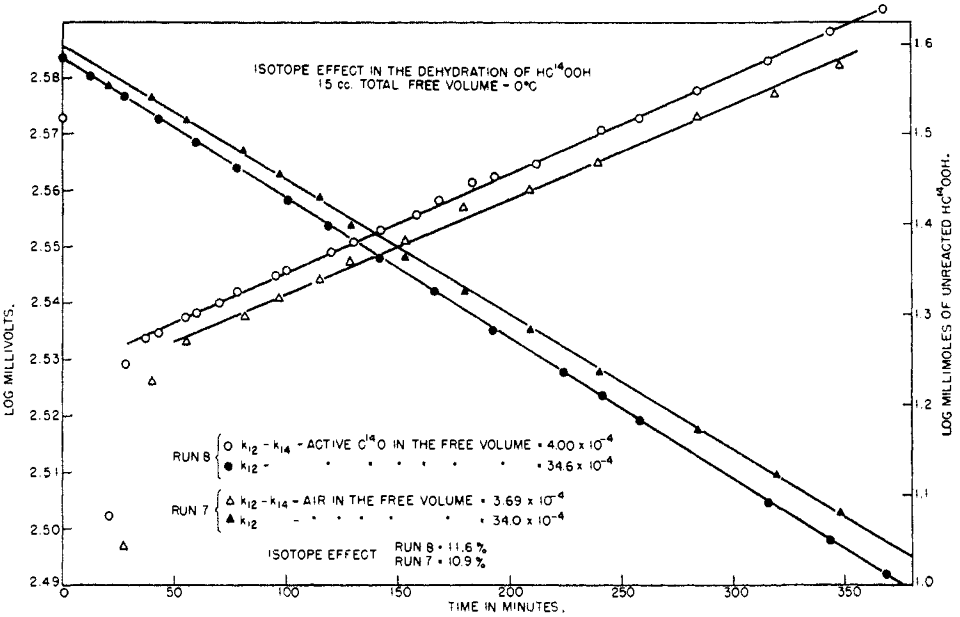

Fig. 2.

mic acid in this range, and (b) other studies showed that all initial air was swept from the system during the first 30% of the reaction.

C. **Supplementary Experiments.**—In order to demonstrate that the observed change in specific activity with time was not due to unknown factors in the flow method of evaluation, a quantity of formic-C^{14} acid azeotrope was decomposed in a system designed to collect successive 300-ml. samples of evolved carbon monoxide. These were transferred individually to an ion chamber for radioactivity determination. An increase in specific activity was found in each successive volume of gas. To prove that this increase in specific activity was not due to the evolution of some inactive gas at a changing rate, the first and tenth samples were burned quantitatively to carbon dioxide, precipitated as barium carbonate and reconverted to carbon dioxide. The specific activity of this gas was determined using the same ion chamber as that used for carbon monoxide. The specific activities of the carbon monoxide and carbon dioxide were in the same ratio for both samples.

Since the flow system initially contained air, which was slowly displaced by evolved carbon monoxide, it was necessary to demonstrate that all of the air was swept out within the first thirty per cent. of reaction. Although the air-elution curves, obtained during each experiment by plotting the per cent. oxygen as registered by the Pauling meter against time, indicated that the air was completely displaced by the first 300 ml., additional proof was sought by passing carbon monoxide of constant specific activity through the flow system which initially contained air. The radioactivity in the chamber was measured as a function of volume of gas passed through. The air-elution curve indicated the complete removal of air by 300 ml. of carbon monoxide.

To rule out the possibility that the measured difference in the isotope effect at two temperatures was affected by the different degrees of internal mixing resulting from the different gas flow rates, two experiments were performed in which the internal mixing was altered or reduced in influence. For these experiments the dead space in the flow system was reduced by 90% by altering the shape of the reaction vessel, and by removal of trap (C), the heat exchanger (B) and the manometer (F). In the first experiment, the run was made at 0° in the normal way with the system filled initially with air. In the second experiment the system was filled before the run with carbon monoxide of higher specific activity than that of the formic acid which was decomposed in the usual way at 0°. As shown in Table I, Runs 7 and 8, respectively, the values of the isotope effect calculated from these modified experiments were not significantly different from values calculated for other runs at the same temperature.

A final experiment was performed to demonstrate that the observed difference in values of the isotope effect at two temperatures was not affected by instrument variation resulting from the effect on the ion chamber of the different gas flow rates. When samples of carbon-C^{14} monoxide were passed through the chamber at several widely different rates, the recorded radioactivities were not affected by the flow through the chamber, if the pressure and temperature were maintained constant.

Calculations and Results

A. **Derivation.**—Since the dehydration of formic acid was shown to be first order under the conditions used in the present work, it was possible to evaluate the isotope effect from the kinetic measurements and the slope of the line obtained by plotting the logarithm of the specific activity of the increment of carbon monoxide volume *versus* the time at which the volume increment was collected:

Let k_{12} = the first-order rate constant for the dehydration of HC^{12}OOH
k_{14} = the corresponding value for HC^{14}OOH
C^{12} = the concentration of HC^{12}OOH remaining at time, t
C_0^{12} = the initial concentration of HC^{12}OOH
C^{14} and C_0^{14} = the corresponding values for HC^{14}OOH
K, K' = constants
S = the specific activity of carbon monoxide (activity per unit volume) being produced at time, t

$$S \propto \frac{\text{Rate of production of C}^{14}\text{O at time, } t}{\text{Sum of production rates of C}^{14}\text{O and C}^{12}\text{O at time } t}$$

$$\therefore S = K\frac{k_{14}C^{14}}{k_{14}C^{14} + k_{12}C^{12}} \cong K\frac{k_{14}C^{14}}{k_{12}C^{12}} \text{ (at tracer level)}$$

$$\therefore S = K\frac{k_{14}C_0^{14}e^{-k_{14}t}}{k_{12}C_0^{12}e^{-k_{12}t}}$$

$$\ln S = K' + (k_{12} - k_{14})t$$

Therefore, $k_{12} - k_{14}$ has the value of 2.303 times the slope if log S is plotted against time. From values of $(k_{12} - k_{14})/k_{12}$, values of k_{14}/k_{12} are readily calculated.

B. **Calculation of the Isotope Effect Values.**—Table I records the values of the isotope effects at four temperatures. For every experiment, the value of $(k_{12} - k_{14})$ obtained was divided by the value of k_{12} obtained for the *same run*. For the experiments at 0° and 24.75° the 95% confidence interval is reported for the mean k_{12} values and for the mean 100 k_{14}/k_{12} values.

TABLE I

VALUES OF THE RATE CONSTANT AND THE ISOTOPE EFFECT AT FOUR TEMPERATURES

Temp., °C.	k_{12}, min.$^{-1}$ × 10^4	$(k_{12} - k_{14})$, min.$^{-1}$ × 10^4	$100k_{14}/k_{12}$
0.00 ± 0.05	34.45	4.247	87.68
	34.29	3.544	89.66
	34.50	4.058	88.24
	33.58	3.556	89.41
	33.99	3.501	89.70
	34.98	3.915	88.81
	33.99	3.692	89.14
	34.59	3.996	88.45
	34.30 ± 0.30		88.89 ± 0.52
14.75 ± 0.05	216	19.2	91.12
	221	21.1	90.46
	219		90.79
18.75 ± 0.05	380	34.6	90.89
	359	31.4	91.25
	370		91.07
24.75 ± 0.05	755.6	65.68	91.31
	733.0	63.79	91.30
	730.3	60.85	91.67
	764.1	58.82	92.30
	763.9	72.77	90.47
	749.3 ± 14.8		91.41 ± 0.58

C. Calculation of the Values of ΔE and E.—From the Arrhenium equations for the dehydration of the two species of formic acid, the following expression was derived

$$\ln \frac{k_{14}}{k_{12}} = \ln \frac{A_{14}}{A_{12}} - \Delta E/RT$$

If it is assumed that A_{14}/A_{12} is constant in the temperature range, 0–25°, ΔE was calculated from the slope of the line obtained by plotting the logarithms of each of the seventeen values of k_{14}/k_{12} against $1/T$,

$$\Delta E = 189 \pm 53 \text{ cal. per mole}$$

The 95% confidence interval of this ΔE value was calculated as twice the standard deviation from the line fitted by the method of least squares. In an exactly analogous manner the seventeen values of k_{12} gave

$$E = 20{,}118 \pm 139 \text{ cal. per mole}^4$$

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[4] *Cf.* E. Schierz, THIS JOURNAL, **44**, 447 (1922); E. Schierz and H. Ward, *ibid.*, **50**, 3240 (1928).

OAK RIDGE, TENNESSEE RECEIVED APRIL 27, 1951

[CONTRIBUTION NO. 533 FROM THE CHEMISTRY LABORATORIES OF INDIANA UNIVERSITY]

Heats of Solution and Reaction in Liquid Ammonia. VIII.

BY HARVEY DALE MULDER[1] WITH FREDERIC C. SCHMIDT

Several heats of solution of inorganic salts have been measured. The heat of neutralization of the ammono base, KNH_2, with the ammonium salts of various strong acids, has been determined and yields a value of 26.1 kcal. This heat effect is much larger than the heat of neutralization of strong acids and bases in water. Also heats of formation of Na_2Se and Na_2Se_2 have been measured directly by means of the liquid ammonia calorimeter.

I. Introduction

In the previous paper[2] there were reported the salts of such bases as the alkyl amines. We have extended these measurements to salts of such organic bases as guanidine and hydroxylamine. Heats of solution of potassium amide over a moderate range of concentrations were made as a comparison with the heats of solution of its analog in water, NaOH.

Kraus[3] made one measurement of the heat of neutralization of the ammono base, potassium amide, with ammonium bromide. We have repeated this work by measuring the heat effect of this base with other ammono acids in liquid ammonia; such as NH_4I, NH_4NO_3, NH_4SCN and NH_4Br. The heat of combination of the ammonium ion with the amide ion is 26,1 kcal., which is lower than the value found by the previous workers.[3]

II. Experimental

The calorimeter and procedure were the same as used in previous measurements.[2] However, a larger pipet was constructed to deliver 46.5 ml. of liquid ammonia at −33° in order that the measurements might be made in more dilute regions than formerly.

The guanidine salts were prepared from guanidine carbonate supplied to us by the Nitrogen Division of the American Cyanamid Company at Stamford, Connecticut. All salts used were recrystallized from water. The sodium and potassium were Baker Analytical Reagent Grade and were put into fragile sample bulbs according to the technique of Kraus and Schmidt.[4]

The potassium amide was prepared by reaction of the metal with ammonia. The potassium had previously been put into fragile sample bulbs so that the amide was

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[1] This paper comprises the greater part of the thesis submitted by Harvey D. Mulder in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Chemistry, Indiana University.

[2] E. Hennelly, D. K. Stevens, M. Warren, H. Zuhr, J. Sottysiak with F. C. Schmidt, THIS JOURNAL, **69**, 1025 (1947).

[3] C. A. Kraus and J. A. Ridderhof, *ibid.*, **56**, 79 (1934).

[4] C. A. Kraus and F. C. Schmidt, *ibid.*, **56**, 2297 (1934).