DEC 1 HOER TC60 0494

Annual Report of the Director of
the Geophysical Lab.
Carnegie Institution of Washington

yearbook 60

(1960-1961).

isotopic forms of formate ion by formic dehydrogenlyase

$$HC^{12}O_2^- + H_2O \xrightarrow{k^{12}} HC^{12}O_3^- + H_2 \qquad (1)$$

$$HC^{13}O_2^- + H_2O \xrightarrow{k^{13}} HC^{13}O_3^- + H_2 \qquad (2)$$

were studied using the partially purified enzyme isolated from *Clostridium* sp. This enzyme was kindly supplied by Dr. Fred Sisler of the U. S. Geological Survey. The C^{13}/C^{12} ratio in the liberated bicarbonate ion after varying percentages of reaction was compared with that after complete reaction. The ratio of specific rate constants, k^{12}/k^{13}, was measured to be 1.0260 at room temperature.

The relative rates of decarboxylating the isotopic forms of *l*-glutamic acid by glutamic decarboxylase

$$\text{HOOC—CH}_2\text{—CH}_2\text{—CHNH}_2\text{—C}^{12}\text{OOH} \xrightarrow{k^{12}} \text{C}^{12}\text{O}_2 + \text{HOOC—CH}_2\text{—CH}_2\text{—CH}_2\text{NH}_2 \qquad (3)$$

$$\text{HOOC—CH}_2\text{—CH}_2\text{—CHNH}_2\text{—C}^{13}\text{OOH} \xrightarrow{k^{13}} \text{C}^{13}\text{O}_2 + \text{HOOC—CH}_2\text{—CH}_2\text{—CH}_2\text{NH}_2 \qquad (4)$$

were studied using the crystalline enzyme obtained from Nutritional Biochemicals Company of Cleveland, Ohio. The ratio of the specific rate constants k^{12}/k^{13} was 1.0259 at room temperature.

These decarboxylations cannot be carried out nonenzymatically, but the results can be compared with other decarboxylations described in the literature. Bigeleisen and Wolfsberg (1958) have reviewed the data for the C^{13} isotope effect in the thermal decarboxylation of malonic, mesitoic, and trichloroacetic acid at high temperatures. When these data, along with some unpublished data from this laboratory on the thermal decarboxylation of alanine, are extrapolated down to room temperature, a value for the ratio of specific rate constants of about 1.04 is obtained. Thus the enzymes used in this experiment are able to carry out a reaction with about half the isotope effect observed for a reaction of this type carried out nonenzymatically.

The theory of kinetic isotope effects specifically predicts that there is no relationship between the activation energy and the isotope effect. The following explanations are proposed. A reaction occurring at a catalytic surface may be formally separated into five steps, the slowest of which will determine the rate and the kinetic isotope effect of the over-all process. The steps are (1) transport of reactants to the active site of the catalyst, (2) adsorption of the reactants on the catalyst, (3) reaction on the surface, (4) desorption of the products, and (5) transport of the reaction products from the surface into the bulk solution. A large isotope effect will occur if the rate-determining step is process 3 with a rupture of a bond to the carbon of the carboxyl group. In enzyme-catalyzed reactions, however, the other steps can be rate determining. They would be expected to have a smaller isotope effect than the actual reaction of the molecule in step 3.

THE PHYSICAL CHEMISTRY OF ISOTOPIC SUBSTANCES

The Effect of Physical Changes on Isotope Fractionation

T. C. Hoering

To make quantitative applications of isotopes in geochemistry it is necessary to have experimental evidence about the effect of temperature, pressure, composition, and other variables on isotope fractionation. Accordingly, measurements have been made on (1) the isotope effect in the solution of CO_2 in water, (2) the effect of varying the activity coefficients of H_2O^{18} and H_2O^{16}, (3) the effect of pressure on isotope fractionation, and (4) the temperature coefficient of an isotope effect. The isotope fractionation factor accompanying a given chemical reaction is, in general, varimarily dependent on the temperature and only secondarily on some of the other physical variables of the system. A notable exception to this generalization is the isotope effect in the vaporization of water.

The fractionation of the oxygen and carbon isotopes between gaseous and aqueous CO_2 was studied by bringing the phases in contact until isotopic equilibrium was established. The two phases were then isolated, and a sample of CO_2 was quantitatively isolated from each. The enzyme carbonic anhydrase was added to several runs to ensure that isotopic equilibrium had been reached. In all experiments the *p*H of the solution was adjusted so that the concentration of bicarbonate ion was negligible. The isotope ratios of the CO_2 from the two phases were compared in the mass spectrometer. The fractionation factor for the reaction

$$C^{12}O_2\,(g) + C^{13}O_2\,(aq) \rightleftarrows C^{13}O_2\,(g) + C^{12}O_2\,(aq) \qquad (1)$$

was found to be 1.0008 at 26.9°C. The fractionation factor for the reaction

$$CO^{16}O^{16}\,(aq) + CO^{16}O^{18}\,(g) \rightleftarrows CO^{16}O^{18}\,(aq) + CO^{16}O^{16}\,(g) \qquad (2)$$

was found to be 1.0018 at 26.9°C. It is interesting to note that the heavy isotope of oxygen and the light isotope of carbon concentrate in the aqueous phase.

The change of the fractionation factor with temperature for the oxygen isotopes in CO_2 and H_2O

$$CO^{16}O^{16}\,(g) + H_2O^{18}\,(l) \rightleftarrows CO^{16}O^{18}\,(g) + H_2O^{16}\,(l) \qquad (3)$$

was measured at four temperatures between 15.0° and 46.0°C. No attempt was made to measure the fractionation factor itself. The temperature coefficient was -0.190 per mil per °C. This corresponds to a change in enthalpy, ΔH°, for reaction 3 of -33.8 cal per mole at 26.9°C. The value of the equilibrium constant for reaction 3 is given in the literature (Webster, Wahl, and Urey, 1935) as 1.041. The standard entropy change, ΔS°, is then -0.0352 cal per degree at 26.9°C.

The equilibrium constant for reaction 3 can be written

$$K = \frac{\left[\dfrac{a'}{a}\right]_{CO_2}}{\left[\dfrac{a'}{a}\right]_{H_2O}} = \frac{\left[\dfrac{\gamma'}{\gamma}\right]_{CO_2} \left[\dfrac{CO^{16}O^{18}}{CO^{16}O^{16}}\right]}{\left[\dfrac{\gamma'}{\gamma}\right]_{H_2O} \left[\dfrac{H_2O^{18}}{H_2O^{16}}\right]} = \Gamma\alpha \qquad (4)$$

The primes indicate molecules with the heavy isotope; *a* is the thermodynamic activity; γ, the activity coefficient; α, the measured isotope fractionation factor. The equilibrium constant is a product of an activity coefficient term, Γ, and a measured fractionation factor. At a fixed temperature and pressure, *K* is a true constant, and changes in the measured fractionation factor reflect changes in the activity coefficient term brought about by physical changes in the system.

In a series of experiments, CO_2 gas was brought into isotopic equilibrium with pure water, with a 10 *M* solution of lithium chloride and with a solution of 30 weight per cent dioxane. Since these solvents are believed to interact only with H_2O and not CO_2, the effects result from changes in the ratio of the activity coefficients of the isotopic forms of water. The isotopic compositions of the oxygen in the CO_2 in the gas phase were compared, and Γ was calculated for the different solutions.

If the value of Γ for the system with pure water is taken to be 1.0000, its value for the dioxane-water mixture is 0.9983 at 25.9°C. It is interesting to note that the equilibrium constant for the exchange of oxygen isotopes between gaseous water and gaseous CO_2 is a factor of 1.008 larger than that for gaseous CO_2 and liquid H_2O. Diluting H_2O with dioxane shifts the oxygen isotope fractionation factor for CO_2–liquid H_2O exchange reaction in the direction of that for CO_2–gaseous H_2O.

The cause of this shift results, in part, from the breakdown of the hydrogen bonded structure of liquid H_2O. The CO_2 is then exchanging with monomeric H_2O molecules rather than the polymeric structure of pure liquid H_2O.

The value of Γ for the concentrated solution of lithium chloride was 1.0008. This is caused by the selective solvation of lithium ions by water slightly enriched in O^{18} leaving the bulk solvent enriched in O^{16}.

Quantitative treatments of geochemical problems involving the isotopic forms of water with the use of isotope fractionation factors should consider activity coefficients. Little is known of the activity of water in supercritical solutions, in silicate melts, or in ore solutions.

Pressure is an important geological variable, and its effect on isotope fractionation factors is of interest in using stable isotopes as geological tracers. A theoretical estimate can be made of the maximum pressure effect for the exchange of oxygen isotopes between gaseous H_2O and gaseous CO_2

$$H_2O^{18}\,(g) + CO^{16}O^{16}\,(g) \rightleftarrows H_2O^{16} + CO^{16}O^{18} \qquad (5)$$

The problem is to determine whether isotopic substitution makes an appreciable change in the volume of a molecule and thus introduces a $P\Delta V$ term to the free energy of a system. The equilibrium constant for reaction 5 is given by

$$K = \frac{[Q^{0\prime}/Q^{0}]_{CO_2}}{[Q^{0\prime}/Q^{0}]_{H_2O}} \qquad (6)$$

where the primes indicate substitution by the heavy isotopes and Q^0 the statistical thermodynamic partition function for the species indicated by the subscript. The ratios of partition functions are assumed to be separable

$$\left[\frac{Q'^0}{Q^0}\right] = \left[\frac{Q'}{Q}\right]_{\text{electronic}} \left[\frac{Q'}{Q}\right]_{\text{translation}} \left[\frac{Q'}{Q}\right]_{\text{rotation}} \left[\frac{Q'}{Q}\right]_{\text{zero point}} \left[\frac{Q'}{Q}\right]_{\text{vibration}} \qquad (7)$$

Consideration of the problem shows that the only term that could be affected significantly by pressure is the ratio of the vibrational partitional functions. If the chemical bonds involving the isotopic substitution are assumed to be harmonic oscillators, the substitution will change the frequency of oscillation but not the amplitude and hence not the volume of the molecule. However, actual chemical bonds are anharmonic oscillators, and the amplitude of vibration and thus the volume of the molecule containing the lighter isotope will be greater than those of the molecule with the heavy isotope. The maximum pressure effect can be estimated by calculating the equilibrium constant for reaction 5, assuming harmonic oscillation of the bonds, and then making the correction for the anharmonicity. The size of the correction will give an estimate of the maximum possible effect of the increased volume of the molecule. This calculation is readily made by the procedure of Bigeleisen and Mayer (1947), and the correction is found to be only 1.001. An examination of tables of spectroscopic constants for those isotopic molecules that have been measured in sufficient details shows that the anharmonicity correction for the CO_2-H_2O exchange will be relatively large in comparison with other pairs, so that the effect of pressure on isotope fractionation factors will, in general, be small and of the order of magnitude of experimental errors.

An experimental verification of this theory, using gaseous CO_2 and gaseous H_2O, is not readily accomplished. Preliminary experiments were made using gaseous CO_2 and liquid H_2O under argon pressures of 4 kb. These experiments were not satisfactory, owing, in part, to the difficulty of obtaining high pressures of argon free of oil from the pumping system. In addition, CO_2 is more soluble under high pressures of an inert gas. This discussion has shown that solution of CO_2 in H_2O produces isotope fractionation. It would be necessary to separate the change in the fractionation factor produced by the enhanced solubility from that caused by changes in pressure.

The effect of pressure on the fractionation of the oxygen isotopes between bicarbonate ion and water was studied:

$$H_2O^{18} + HCO_3^{16-} \rightleftharpoons H_2O^{16} + HCO_2^{16}O^{18-} \qquad (8)$$

A solution of 0.1 M potassium bicarbonate, pH 8.4, was sealed in polyethylene tubing, placed in a high-pressure bomb, and filled with water to a pressure of 4 kb. Blank experiments with solutions of colored dyes showed that polyethylene was satisfactory for this purpose. At this pH the exchanging species in solution is predominantly the bicarbonate ion in equilibrium with only small amounts of carbonate ion and dissolved carbon dioxide. The effect of the high pressure is to shift the equilibrium slightly in the direction of carbonate ion, but the amount of this shift will have a negligible effect on these experiments. The high-pressure bombs, along with identical samples at 1 atmosphere pressure, were placed in a thermostat at 43.5°C for a period known to be greater than 10 half-lives of the exchange reaction. The samples were then precipitated with an excess of barium hydroxide, centrifuged, washed, and dried. No isotope exchange is induced by this separation process. Carbon dioxide was generated from the barium carbonate by 100 per cent phosphoric acid, and the oxygen isotope ratios of the CO_2 from the high-pressure samples were compared with those of the CO_2 from samples equilibrated at 1 atmosphere. The precision of the separation and isotopic analysis was found to be ±0.2 per mil change in the O^{18}–O^{16} ratio. The average results of three high-pressure experiments differed from the atmospheric-pressure experiments by 0.2 ± 0.2 per mil in the oxygen isotope ratio.

These experiments indicate that pressure is not an important variable in determining the isotopic composition of substances.

The Carbon Isotope Effect in the Synthesis of Diamond

T. C. Hoering

When diamond is synthesized from graphite in the laboratory, there could be either the equilibrium among the isotopes

$$C^{12}(\text{diamond}) + C^{13}(\text{graphite}) \rightleftharpoons C^{13}(\text{diamond}) + C^{12}(\text{graphite}) \qquad (1)$$

or a competition in the rates among the isotopes

$$C^{12}(\text{graphite}) \xrightarrow{k^{12}} C^{12}(\text{diamond}) \qquad (2)$$

$$C^{13}(\text{graphite}) \xrightarrow{k^{13}} C^{13}(\text{diamond}) \qquad (3)$$

At the high temperature at which this synthesis was carried out, it would be predicted that there would not be a measurable isotope effect either in rates or in equilibrium. Since the reaction is carried out at a very high pressure, however, and since the effect of pressure on isotope effects is unknown, it was considered worth while to carry out the following experiment.

Diamond was synthesized from graphite by Boyd and England in this Laboratory at a pressure of 70 kb and at a temperature higher than 1700°C with nickel as the metal catalyst.

Part of the graphite rod used in the bomb and the diamond isolated from the run were combusted to CO_2. Preliminary experiments showed that graphite could be combusted with a reproducibility of better than 0.1 per mil difference in the C^{13}/C^{12} ratio, and experiments on commercial cutting diamonds showed a reproducibility of combustion of diamond of about 0.1 per mil.

The fractionation between the graphite and diamond phases measured only 0.3 per mil difference in the C^{13}/C^{12} ratio (a factor of 1.0003). Since this is only about twice the estimated experimental error, there appears to be little, if any, effect of pressure at this high temperature.