[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF NEW MEXICO]

The Kinetics of the Exchange of Iodine between Diiodotyrosine and Molecular Iodine in Aqueous Solution

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A measurable exchange has been observed in aqueous solutions between iodine and diiodotyrosine. The exchange reaction was investigated over a temperature range from 20.5 to 42.1° and a *p*H range from 1.33 to 5.25. The diiodotyrosine concentration varied from 4.76×10^{-5} to 1.461×10^{-2} *M* and that of the iodine from 1.03×10^{-5} to 3.95×10^{-4} *M*. The rate law for the exchange reaction is $R = 7.87 \times 10^{13} e^{-23060/RT}(C_9H_9NO_3I_2)(I_2)/(H^+)$, where the units of R are moles $\times$ liter^{-1} $\times$ sec.$^{-1}$. No exchange was observed between potassium iodide and diiodotyrosine in the presence of sodium sulfite over a period of two weeks at 62.8°. Also, no exchange was observed between diiodotyrosine and iodine or potassium iodide in methanol or ethanol at 52° over a 24-hour period.

Introduction

Miller, *et al.*,[2] reported that the exchange of iodine between diiodotyrosine and molecular iodine in aqueous solution proceeds at a measurable rate at 25°. We have investigated the kinetics of this reaction and found the rate of exchange to be directly proportional to the concentrations of iodine and diiodotyrosine and inversely proportional to the hydrogen ion concentration.

No measurable exchange was observed between iodide ion and diiodotyrosine in agreement with the results reported by Süe and LeBlond.[3]

Experimental

Radioactivity.—The 8.0-day I^{131}, which was used as tracer in all experiments, was extracted from neutron-irradiated tellurium metal according to the procedure of Ballantine and Cohn.[4] The basic sulfite solution of active iodine was evaporated to 10 ml., acidified with sulfuric acid, treated with an excess of potassium iodate and finally extracted with 10 ml. of benzene. A stock solution of active potassium iodide was obtained by shaking 10 ml. of 0.00015 *M* potassium iodide with the benzene solution of active iodine. The active aqueous solution was washed with several portions of pure benzene. A stock solution of active iodine in benzene was prepared by shaking a portion of the aqueous active potassium iodide solution with 5 ml. of benzene containing 50 mg. of iodine. This active benzene solution was subsequently washed with an acid sulfate buffer.

The radiochemical purity of the tracer was checked by observing its decay rate. The active sample decayed with an 8.0-day half-life over a period of five half-lives in satisfactory agreement with the previously reported value.[5]

Chemicals.—Diiodotyrosine was synthesized from Eastman Kodak Co. L(−)tyrosine according to the method of Wheeler and Jamieson.[6] Analysis of the final product according to the method of Carius[7] yielded 99.44% diiodotyrosine on the basis of the iodine content. Analytical Reagent Grade chemicals were used throughout without further purification.

Procedure.—Buffered solutions of diiodotyrosine and active iodine were brought to reaction temperature and 5-ml. aliquots were transferred to reaction vessels which were then placed in a light-proof constant temperature (±0.05°) water-bath. After a given time had elapsed the reactants were separated and the activity associated with the diiodotyrosine and iodine determined. The *p*H of the reaction mixtures ranged from 1.33 to 2.52 and 3.52 to 5.25 and was maintained by use of the sodium sulfate–sulfuric acid and sodium acetate–acetic acid systems, respectively. The ionic strength of all solutions was adjusted to 0.1 by addition of sodium sulfate.

Solutions of diiodotyrosine and iodine were prepared by dissolving a known amount of the solids in appropriate volumes of buffer. The concentrations of the diiodotyrosine in the reaction mixtures were computed from the known concentrations of the diiodotyrosine stock solutions. The concentration of iodine in a reaction mixture was determined by extracting the iodine into benzene and subsequently measuring the optical density of the benzene solution in a Beckman DU spectrophotometer. Iodine activity extracted into a buffered solution from the stock solution of I^{131} in benzene was used to inoculate the iodine solutions with I^{131} activity. The *p*H of a reaction mixture was determined with the aid of a Beckman model G *p*H meter.

In the experiments which involved the exchange of iodine between potassium iodide and diiodotyrosine 10-ml. aliquots were withdrawn at various times from the reaction mixture. The concentrations of potassium iodide in the reaction mixtures were calculated from the known concentration of a stock solution of potassium iodide which was analyzed according to the procedure of Kolthoff and Sandell.[8] Zero time, in each instance, was taken as the time of mixing of the solutions.

Separation Procedure.—Iodine was separated from diiodotyrosine *via* extraction with benzene. Five milliliters of the reaction mixture was shaken with two 5-ml. portions of benzene; the layers were centrifuged apart. Experiments with tagged (I^{131}) diiodotyrosine showed that less than 0.1% of the diiodotyrosine accompanied the iodine in this separation procedure. Experiments in the absence of diiodotyrosine showed that the amount of iodine activity which remained in the aqueous phase was greater than that predicted from the hydrolysis of iodine[9] and, therefore, it was necessary to apply a small correction to the activity in the aqueous phase. This activity exchanged rapidly with molecular iodine and, in general, did not exceed 5% of the total iodine activity.

Separation of potassium iodide from diiodotyrosine was effected by oxidation with iodate in the presence of acid; the iodine formed was extracted with carbon tetrachloride. Two milliliters of 0.047 *M* potassium iodate was added to 10 ml. of the reaction mixture which was then extracted with two 5-ml. portions of carbon tetrachloride; the phases were centrifuged apart. In this separation less than 0.5% of the diiodotyrosine accompanied the iodine.

Measurement of Radioactivity.—The energetic gamma radiation associated with the decay of I^{131} was detected with a Technical Associates decade-type scaler equipped with a Radiation Laboratories Geiger–Müller tube which was enclosed in a $^{1}/_{16}$ inch lead shield. Liquid samples were transferred to annular glass vessels which were then placed over the Geiger–Müller tube.

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[1] This paper is a portion of the dissertation presented by Alfred H. Zeltmann in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Graduate School of the University of New Mexico, June, 1952.

[2] W. H. Miller, G. W. Anderson, R. K. Madison and D. J. Salley, *Science*, **100**, 340 (1944).

[3] P. Süe, *Compt. rend.*, **212**, 237 (1941); C. P. LeBlond and P. Süe, *Am. J. Physiol.*, **134**, 549 (1941).

[4] D. S. Ballantine and W. E. Cohn, "The Preparation of Carrier-Free I^{131}," United States Atomic Energy Commission Report-MDDC-1600.

[5] J. J. Livingood and G. T. Seaborg, *Phys. Rev.*, **54**, 775 (1938).

[6] H. L. Wheeler and C. S. Jamieson, *Am. Chem. J.*, **33**, 365 (1905).

[7] F. P. Treadwell and W. T. Hall, "Analytical Chemistry," Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1947, p. 293.

[8] I. M. Kolthoff and E. B. Sandell, "Textbook of Quantitative Inorganic Analysis," The Macmillan Co., New York, N. Y., 1948, p. 629.

[9] W. C. Bray and E. L. Connolly, THIS JOURNAL, **33**, 1485 (1911).

Results and Discussion

The reaction investigated is $C_9H_9NO_3I_2 + II^* = C_9H_9NO_3II^* + I_2$, where the asterisks indicate radioactive atoms. The logarithmic form of the first-order exchange law[10] for this particular reaction is

$$-\ln(1 - F) = [(C_9H_9NO_3I_2) + (I_2)]Rt/2(C_9H_9NO_3I_2)(I_2) \quad (1)$$

where $(C_9H_9NO_3I_2)$ = total concentration (active + inactive) of diiodotyrosine in moles per liter; (I_2) = total concentration (active + inactive) of iodine in moles per liter; F = fraction exchange at time t; R = actual rate at which the exchange of iodine atoms between diiodotyrosine and iodine takes place. Since in any one experiment $(C_9H_9NO_3I_2)$ and (I_2) are known it is possible to evaluate R, the rate of exchange, for the particular experiment after having determined $T_{1/2}$, the half-time for exchange, from a plot of log $(1 - F)$ *vs.* t. This type of plot, according to equation 1 should give a straight line passing through the origin at zero time regardless of the mechanism. Such plots were obtained which extrapolate to zero fraction exchange at zero time as is required by the first-order exchange law.

Dependence on Iodine and Diiodotyrosine.—If the reaction is first order with respect to both the iodine and diiodotyrosine concentrations, $R = k_1(I_2)(C_9H_9NO_3I_2)$, and a plot of $R/(C_9H_9NO_3I_2)$ *vs.* (I_2) should yield a straight line of slope equal to k_1 and of zero intercept. The data obtained at an average pH of 2.51 are plotted in Fig. 1 and suggest that the rate of exchange is first order with respect to both the iodine and diiodotyrosine concentrations.

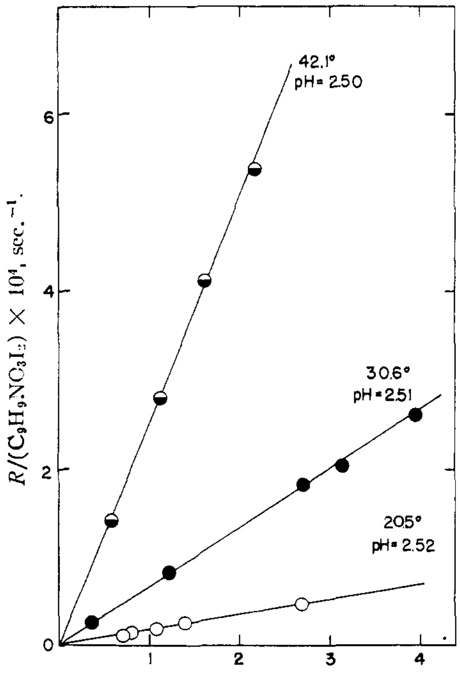

Fig. 1.—Dependence of the ratio $R/(C_9H_9NO_3I_2)$ on the iodine concentration.

Dependence on the Hydrogen Ion Concentration.—Preliminary experiments indicated that the rate of exchange was dependent on the pH of the reaction mixture. Assuming that $R = k_2(C_9H_9NO_3I_2)\cdot(I_2)(H^+)^n$, a plot of log $R/(C_9H_9NO_3I_2)(I_2)$ *vs.* pH should be linear with a slope equal to $-n$. The data are plotted in Fig. 2. It is seen that the plots are linear suggesting a simple dependence on a power of the hydrogen ion concentration. The slope in each instance is 0.997 ± 0.007 indicating that the rate of exchange, R, is inversely proportional to the hydrogen ion concentration.

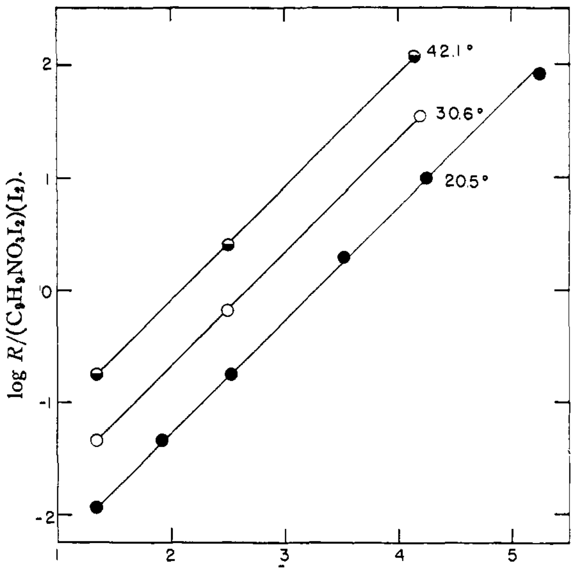

Fig. 2.—Dependence of the exchange reaction on the hydrogen ion concentration.

Effect of Light on the Rate of Exchange.—The result of an experiment carried out in daylight at 20.5° at a pH of 2.52 indicates that light has an accelerating effect on the rate of the exchange reaction.

Exchange between Diiodotyrosine and Potassium Iodide.—Half-times of the order of one hundred minutes were obtained for the exchange reaction between diiodotyrosine and potassium iodide at 62.8° in the pH range from 2.46 to 6.30. The concentration, in all instances, of potassium iodide was 2.95×10^{-4} M and that of diiodotyrosine 6.81×10^{-4} M. Plots of ln $(1 - F)$ *vs.* t were not linear and indicated an induction period from 5 to 60 minutes which was also observed by Miller, *et al.*[2] Very probably this induction period and eventual measurable exchange resulted from the slow formation of iodine which was detected visually in benzene extracts of the reaction mixtures. Exchange experiments similar to those mentioned above were carried out in the presence of 0.002 M sodium sulfite and no exchange was detected over a period of two weeks. The iodine probably formed through decomposition of diiodotyrosine since reaction mixtures prepared from stock solutions which had been alternately frozen and thawed under vacuum to

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[10] G. Friedlander and J. W. Kennedy, "Introduction to Radiochemistry," John Wiley and Sons, Inc., New York, N. Y., 1949, p. 285.

remove oxygen yielded results similar to the exchange experiments mentioned above.

Effect of Solvent.—No exchange was observed between diiodotyrosine and iodine or potassium iodide in methanol or ethanol at 52° over a 24-hour period. The diiodotyrosine concentration in each instance was 0.00251 *M*, the iodine concentration was 0.06 *M* and the potassium iodide concentration, 0.05 *M*.

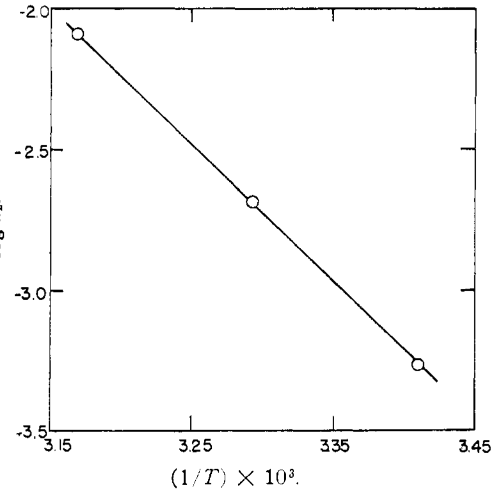


Fig. 3.—The temperature dependence of the exchange reaction.

The Activation Energy.—The curve shown in Fig. 3 is a semi-logarithmic plot of the average values for the velocity constant k_2 (see Table I) against the reciprocal of the absolute temperature. The experimental activation energy is 23,060 ± 300 cal.

TABLE I

AVERAGE VALUES FOR THE VELOCITY CONSTANT k_2 AT VARIOUS TEMPERATURES

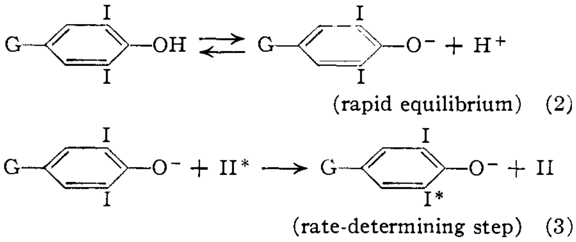
Temp., °C.	$k_2 \times 10^4$, sec.$^{-1}$
20.5	5.33
30.6	20.7
42.1	81.1

The Rate Law.—From the foregoing discussion it appears that the rate of exchange is directly proportional to both the diiodotyrosine and iodine concentrations and inversely proportional to the hydrogen ion concentration over a temperature range from 20.5 to 42.1° and a *p*H range from 1.33 to 5.25. The diiodotyrosine concentration varied from 4.76 × 10^{-5} to 1.461 × 10^{-2} *M* and that of the iodine from 1.03 × 10^{-5} to 3.96 × 10^{-4} *M*. The rate law for the exchange reaction is

$$R = 7.87 \times 10^{13}e^{-23060/RT}(C_9H_9NO_3I_2)(I_2)/(H^+)$$

where the units of *R* are moles × liter^{-1} × sec.$^{-1}$.

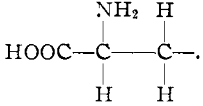
The Mechanism.—A mechanism which is consistent with the experimentally determined rate law is represented by equations 2 and 3



(rapid equilibrium) (2)

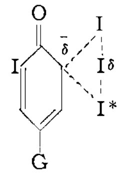
(rate-determining step) (3)

where G represents the para substituent group,



$$HOOC-\underset{H}{\overset{NH_2}{C}}-\underset{H}{\overset{H}{C}}-.$$

A possible activated complex is depicted in the resonance form I below wherein the three iodine atoms are linear and perpendicular to the plane of



I

the ring. This linear arrangement is analogous to the structure of the triiodide ion.[11] Resonance forms in which the benzenoid resonance is maintained are not included because they are quantum-mechanically unacceptable.[12]

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[11] L. Pauling, "The Nature of the Chemical Bond," Cornell University Press, Ithaca, N. Y., 1940, p. **111**.

[12] J. F. Bunnett and R. E. Zahler, *Chem. Revs.*, **49**, 273 (1951).