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DAVIS, CALIFORNIA

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF ILLINOIS INSTITUTE OF TECHNOLOGY]

Kinetics of the Raney Nickel Catalyzed Hydrogenation of Crotonic Acid

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The kinetics of the Raney nickel-catalyzed hydrogenation of crotonic acid in ethanol solution have been studied using an isobaric hydrogenation apparatus. It was found that the rate could be expressed accurately by a Langmuir-type equation of the form: $-dc/dt = [kaAPc/[V(1 + bc)]]\{1/[1 + r(c_0 - c)]\}$, where V is the volume of solvent; c is the concentration of acceptor; A is the surface area of catalyst; P is the hydrogen pressure; k is the specific rate constant; a and b are adsorption coefficients for acceptor; and r is the coefficient for retardation by product. The kinetics appear zero order with respect to crotonic acid for the initial portion of the hydrogenation and first order during the final stages. The reaction is retarded by the product, butyric acid. The kinetic data may be interpreted satisfactorily by a Balandin type dual-site mechanism.

Introduction

The catalytic hydrogenation of a carbon–carbon double bond has often been assumed to follow zero-order kinetics with respect to the hydrogen acceptor.[2–6] However there are several examples of hydrogenations which appear to follow intermediate or first-order rate laws.[7–11] In the case of the hydrogenation of crotonic acid, Lebedev, *et al.*,[12] and Maxted[13] reported zero order, while Fokin[14] found first-order behavior. Balandin,[15] in attempting to reconcile these differences, postulated a rate law based on non-competitive adsorption of the reactants on two types of active centers. The present study was undertaken to obtain precise kinetic data on the hydrogenation of crotonic acid over Raney nickel catalyst in order to examine the validity of the several plausible adsorption mechanisms for catalytic hydrogenation.

Experimental

Raney Nickel Catalyst—Modified Form.—The leaching and digestion procedures of Adkins and Billica[16] were followed using 125 g. of Raney alloy. The alkaline solution was decanted and the catalyst washed 30 times with 1-l. portions of water.

A 2-l. portion of water was added to the catalyst which was held at 100° for 3–4 hr. with vigorous stirring during the last $^1/_2$ hr. to remove excess hydrogen. After decantation, the catalyst was washed 6 times with 500-ml. portions of ethanol, and stored under absolute ethanol in a glass stoppered bottle.

The basis for measurement of the amount of catalyst was its volume after a 5-min. centrifugation.[17]

Although the procedure was followed rigorously, it was impossible to duplicate precisely the activities of the catalysts in separate preparations. It was thus necessary that each of a series of related experiments be made using the same batch of catalyst. Due to the instability of the catalyst, series were completed in 1–2 days, during which time the reproducibility of the kinetic data was satisfactory.

The surface area of a typical catalyst was determined by the adsorption method[18] using myristic acid; the surface area of a typical batch was 30 m.^{2}/g. Using a similar technique it was found that crotonic acid was adsorbed in multilayers. The adsorption isotherm was of the Freundlich type.

Materials.—Tank hydrogen was passed through a "Deoxo" purifier. Absolute ethanol was used: n^{25}D 1.3592. *trans*-Crotonic acid (Tennessee-Eastman Co.) was purified by two crystallizations from light-boiling petroleum ether. The crotonic acid was obtained as white needles, m.p. 72–72.5° (uncor.); lit. 72°. By electrometric titration, the neutralization equivalent was 86.7; theoretical 86.1. Fractional distillation of the product mixture yielded butyric acid: b.p. 163°, n^{20}D 1.3987. Neutralization of the cold, filtered product mixture with NaOH indicated a butyric acid yield of 97.5% based on weight of sodium butyrate recovered. No evidence for esterification was found.

The Isobaric Hydrogenation Apparatus. (Fig. 1).—A 4-l. tank (A), from a Parr hydrogenation apparatus, used as a hydrogen reservoir, was connected to an open-end Hg manometer (B) (Merriam Co.) and to a solenoid valve (C) (General Controls Co.). The solenoid valve was waxed into a Pyrex manifold, which included a silica-gel trap (S), a 500-ml. ballast bulb (D), a mercury manostat (E), a manometer (F), and the reaction bottle (G) (a 250-ml. suction flask). The reaction flask was connected to the apparatus by Tygon tubing. Contacts (H) from the manostat led to the input of a thyratron circuit connected to the solenoid (J). The shaking carriage, in which the reaction flask was mounted, was taken from a Parr apparatus, and could be operated at 260 or 380 cycles/min. The greater part of the flask was immersed in a thermostat.

The flask was fitted with a "breaking tube" (see insert). A Pyrex tube (K), with a thin bottom, was attached to the neoprene stopper (L). Through the brass sleeve (M) a movable brass rod (N) with a neoprene gasket (O) was held against the lower end of the brass sleeve by spring (P). Pressure exerted at the top of the brass rod forces it downward, breaking tube (K). When the pressure on rod (N) is released, the spring forces it up and the gasket is again seated against the bottom of sleeve (M). An inert fluorocarbon grease, BFE-3 (Carbide and Carbon Chem. Corp.)

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(1) Taken from a Ph.D. thesis submitted to the Graduate School of Illinois Institute of Technology.

(2) M. G. Vavon, *Compt. rend.*, **152**, 1675 (1911).

(3) R. Willstätter, *Ber.*, **45**, 1471 (1912).

(4) S. V. Lebedev, *J. Russ. Phys. Chem. Soc.*, **48**, 1002 (1916).

(5) J. S. Salkind, *ibid.*, **52**, 191 (1920); *C. A.*, **17**, 1453 (1923).

(6) E. F. Armstrong and T. P. Hilditch, *Proc. Roy. Soc.* (*London*), **A98**, 27 (1920).

(7) A. A. Zinovyev, *Zhur. Priklad. Khim.*, **23**, 99 (1950); *C. A.*, **44**, 4320 (1950).

(8) S. Ueno and S. Tsuda, *J. Soc. Chem. Ind. Japan*, **46**, 481 (1943).

(9) A. Kailan and F. Hartel, *Monatsh.*, **70**, 329 (1937).

(10) A. Kailan and O. Albert, *ibid.*, **72**, 169 (1938).

(11) F. A. Vandenheuvel, *Anal. Chem.*, **24**, 847 (1952).

(12) S. V. Lebedev, G. G. Kobliansky and A. A. Yabulchik, *J. Chem. Soc.*, 417 (1925).

(13) E. B. Maxted, "Advances in Catalysis," Vol. III, Interscience Publ., Inc., New York, N. Y., 1951.

(14) S. Fokin, *J. Russ. Phys. Chem. Soc.*, **40**, 276, 309 (1908); *C. A.*, **2**, 2896 (1908).

(15) A. A. Balandin, *Bull. acad. sci. U.R.S.S., Classe sci. chem.*, 339 (1945).

(16) H. Adkins and H. R. Billica, THIS JOURNAL, **70**, 695 (1948).

(17) D. R. Levering, F. L. Morritz and E. Lieber, *ibid.*, **72**, 1190 (1950).

(18) H. A. Smith and J. F. Fuzek, *ibid.*, **68**, 229 (1946).

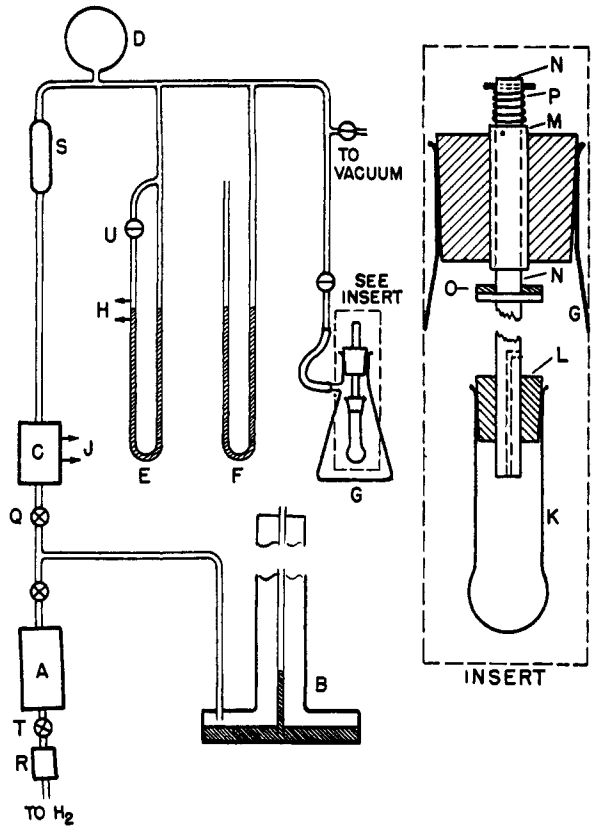

Fig. 1.—The isobaric hydrogenation apparatus.

made a gas tight seal between the rod and the sleeve during the brief period of breaking tube (K). The amount of hydrogen allowed to enter the system during any single activation of the solenoid valve is controlled by the needle valve (Q).

With stopcock (U) open, hydrogen is admitted to the desired pressure; then the by-pass stopcock is closed. The catalyst in ethanol is first saturated with hydrogen at the working pressure by shaking for several minutes. The kinetic run begins when tube K, containing the crotonic acid wet with ethanol, is broken, and the agitation started again. Absorption of hydrogen in the reaction flask (G) will now break the contacts in the manostat, activating the thyratron circuit, opening the solenoid valve which admits a small pulse of hydrogen to the system.

The apparatus was calibrated by allowing hydrogen from the manostated glass system to displace 1 liter of oil. The average number of moles of hydrogen corresponding to 1 cm. Hg drop in pressure of the reservoir at 25° was 2.39×10^{-3}. Quantitative hydrogenations of cyclohexene, maleic acid and crotonic acid gave independent calibration values with an average of $(2.39 \pm 0.02) \times 10^{-3}$ moles H_2/cm. Hg.

An increase in the shaking rate from 260 to 380 cycles/min. did not alter the kinetic curves. It was therefore assumed that at the normal rate of 260 cycles/min. mixing is adequate. Unless otherwise specified, all runs were carried out at 25.0 ± 0.5°.

Experimental Results

From a consideration of general principles it was possible to formulate a rate law within the framework of which the experimental data could be fitted. It is convenient to introduce here the final expression

$$-\frac{dc}{dt} = \left[\frac{kaAPc}{V(1+bc)}\right]\left[\frac{1}{1+r(c_0-c)}\right] \qquad (1)$$

where V is the volume (l.) of solvent; t is the time (min.); A is the volume (ml.) of centrifuged catalyst (assumed proportional to the surface area); P is the pressure (atm.) of hydrogen; c_0 is the initial concentration of the hydrogen acceptor (moles/l.); c is the concentration at time t. Here r, a and b are adsorption constants (l./mole), and k the reaction rate constant, (moles) [(min.) (atm.) (ml. catalyst)]$^{-1}$. Since it was not possible to determine k and a separately, only the product, ka, in (l.) [(min.) (atm.) (ml. catalyst)]$^{-1}$ is obtained.

The validity of eq. 1 is established experimentally by showing that ka is constant at a given temperature and is therefore independent of the initial concentration of hydrogen acceptor, the volume of solution, the hydrogen pressure and the quantity of catalyst. Agreement between the experimental points and the theoretical curves based on eq. 1 was excellent throughout.

Effect of Acceptor Concentration.—This variable was studied first by varying the amount of acceptor while the amount of solvent and other variables were kept constant. Figure 2 displays the effect of varying the concentration of crotonic acid at constant volume of ethanol for a typical batch of catalyst. The horizontal bars on the right margin indicate the stoichiometric end-points. The accompanying circles are experimental end-points measured at a large time (not on the scale). Table I shows the constancy of the calculated ka for seven series of experiments.

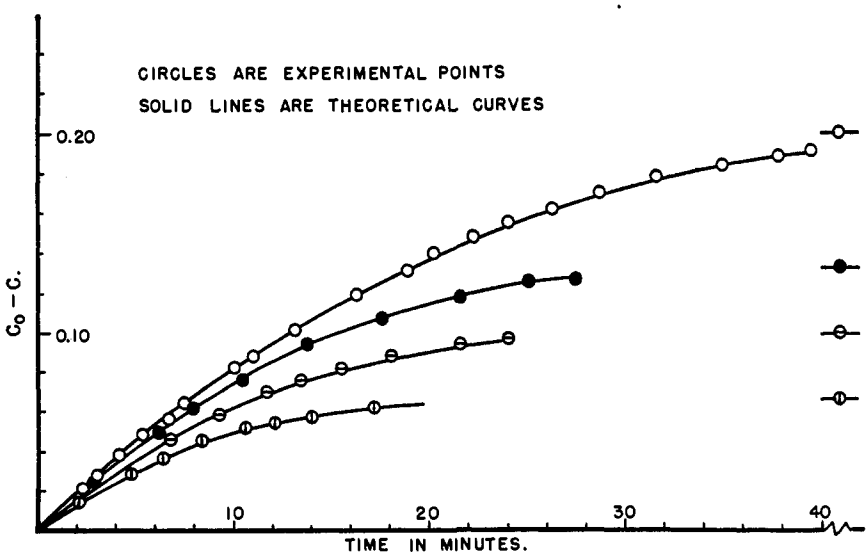

Fig. 2.—The effect of varying initial concentration with volume constant.

The second method of varying concentration was to keep the amount of crotonic acid and other variables constant while the volume of ethanol was varied. Table II summarizes four series of experi-

TABLE I

THE DEPENDENCE OF THE RATE UPON ACCEPTOR CONCENTRATION AT CONSTANT VOLUME OF SOLVENT

Catalyst, Batch	Ml.	Vol., l.	P, atm.	c_0, mole/l.	r, l./mole	ka
A	3.5	0.100	1.45	0.180	1.3	0.029
				.228		.027
				.246		.028
				.310		.029
				.371		.027
B	1.7	.100	1.47	0.161	2.4	0.042
				.346		.046
				.396		.047
C	1.1	.100	1.48	0.134	1.5	0.041
				.172		.043
				.236		.038
				.409		.037
D	1.1	.150	1.48	0.067	4.4	0.025
				.100		.023
				.133		.025
				.200		.023
D	1.1	.100	1.48	0.100	4.4	0.027
				.150		.024
				.200		.026
D	1.1	.050	1.48	0.200	4.4	0.028
				.300		.028
				.400		.026
				.600		.028
E	1.1	.100	1.45	0.310	2.0	0.050
				.172		.043

ments, showing the constancy of ka as the concentration of acceptor is changed.

TABLE II

THE DEPENDENCE OF THE RATE UPON THE VOLUME OF SOLVENT WITH CONSTANT QUANTITY OF ACCEPTOR

Catalyst Batch	Ml.	Vol., l.	P, atm.	c_0, mole/l.	r, l./mole	ka
C	1.1	0.050	1.48	0.344	1.5	0.044
		.100		.172		.043
		.150		.115		.041
D	1.1	0.050	1.48	0.200	4.4	0.028
		.100		.100		.027
		.150		.067		.025
D	1.1	0.050	1.48	0.300	4.4	0.028
		.100		.150		.024
		.150		.100		.023
D	1.1	0.050	1.48	0.400	4.4	0.026
		.100		.200		.026
		.150		.133		.025

Another series of experiments involving concentration was carried out in which all initial conditions were constant except for the amount of crotonic acid, which was varied over a hundred-fold range. The specific initial slopes, Z, defined as (moles) [(min.) (atm.)(ml. catalyst) (liter)]$^{-1}$ at zero time, are summarized in Table III for initial concentrations ranging from 0.030 to 3.00 M.

TABLE III

THE EFFECT OF ACCEPTOR CONCENTRATION UPON THE INITIAL RATE[a]

c_0, mole/l.	Z
0.030	0.0049
.060	.0043
.300	.0086
1.000	.0096
2.000	.0095
3.000	.0096

[a] One ml. of catalyst D was used in each experiment.

Effect of Hydrogen Pressure.—The constants for four series are summarized in Table IV.

TABLE IV

THE DEPENDENCE UPON PRESSURE OF HYDROGEN

Catalyst Batch	Ml.	P, atm.	c_0, mole/l.	r, l./mole	ka	Z
F	3.5	0.85	0.313	1.0	0.035	0.013
		1.17			.036	.014
		1.46			.035	.014
G	1.3	0.74	.257	4.0	0.039	0.014
		1.05			.034	.014
		1.47			.034	.015
C	1.1	0.75	.172	1.5	0.053	0.015
		1.00			.047	.017
		1.48			.034	.017
		1.48			.043	.015
		1.76			.036	.015
D	1.1	0.74	.200	4.4	0.034	0.011
		0.94			.029	.009
		1.48			.026	.009
		1.75			.025	.008

Effect of Amount of Catalyst.—The calculated values of ka are shown in Table V for four series.

TABLE V

THE DEPENDENCE UPON THE AMOUNT OF CATALYST

Catalyst Batch	Ml.	P, atm.	c_0, mole/l.	r, l./mole	ka	Z
F	0.65	1.46	0.313	1.0	0.027	0.012
	1.3				.033	.015
	2.0				.045	.019
	3.5				.035	.014
	3.5				.037	.017
	3.5				.039	.015
	5.4				.032	.012
E	1.05	1.45	.310	2.0	0.052	0.025
	1.9				.044	.018
	3.2				.039	.014
	4.9				.028	.010
	5.9				.033	.012
C	0.7	1.48	.172	1.5	0.033	0.013
	1.5				.043	.013
	2.5				.039	.014
	4.1				.027	.009
D	0.75	1.48	.200	4.4	0.023	0.007
	1.1				.026	.011
	2.6				.034	.011
	4.0				.032	.010
	7.3				.021	.006

Retardation by Product.—The retardation by the product, butyric acid, is demonstrated by the series of experiments shown in Fig. 3. Run 163 of Fig. 3 shows the hydrogenation of 0.0376 mole of crotonic acid in 100 ml. of ethanol (1.7 ml. of catalyst B, hydrogen pressure 1.47 atm.). Run 164 represents the hydrogenation, under the same conditions, of 0.0125 mole of crotonic acid to which has

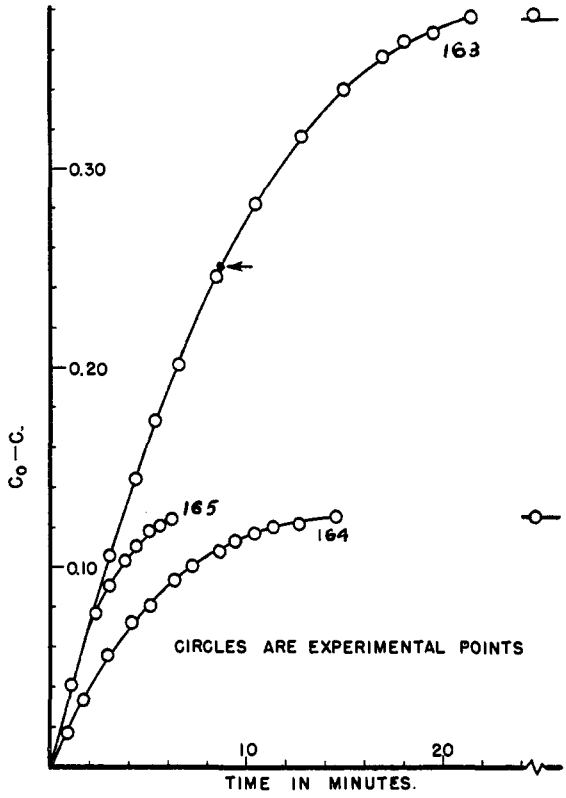

Fig. 3.—The retarding effect of butyric acid.

been added 0.0251 mole of butyric acid. The initial conditions of run 164 were thus identical to those of run 163 at the point indicated by the arrow. Run 165 represents the hydrogenation of 0.0125 mole of crotonic acid with no added butyric acid. Comparison of runs 164 and 165 gives a direct indication of the retarding effect of butyric acid. When the origin of Fig. 3 is translated so that it coincides with that point of run 163 where 0.0125 mole of crotonic acid remained unreacted, the two curves coincide.

Effect of Temperature.—The data are summarized in Table VI. The apparent energies of activation evaluated from the Arrhenius plot of the two sets using catalyst H of Table VI are 5.6 and 5.0 kcal./mole, respectively. For catalyst G the value obtained was 7.4 kcal./mole.

TABLE VI

THE EFFECT OF TEMPERATURE

Batch	Catalyst Ml.	T, °K.	c_0, mole/l.	P, atm.	Z	ka[a]
H	1.7	284	0.129	1.48	0.0080	0.021
		284			.0083	.022
		298			.014	.037
		298			.015	.040
		314.5			.022	.059
		314.5			.023	.061
H	1.7	284	.232	1.48	0.011	0.026
		298			.017	.040
		314.5			.025	.058
G	1.3	288	.256	1.48	0.006	0.014
		308			.014	.032

[a] Calculated from the specific initial slopes.

Discussion

The retardation by butyric acid was quantitatively evaluated by experiments at constant conditions with variable initial concentration of crotonic acid. Rates, R_c, in (moles) [(liters) (min.)]$^{-1}$, were measured graphically and plotted as a function of c. Figure 4 is the differential plot of a typical set. Intersections of vertical lines through this family of curves give the rates, R_c, at constant concentration of crotonic acid and at varying concentrations of butyric acid. The results could be expressed

$$R_0 = R_{c_0}\left[\frac{1}{1 + r(c_0 - c)}\right]$$

The term r is the coefficient for the adsorption of butyric acid.

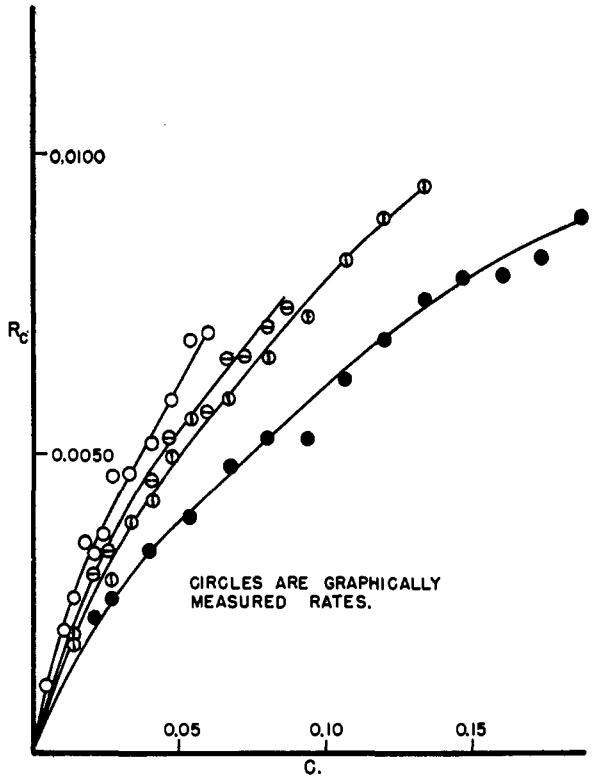

Fig. 4.—The differential analysis of the data of Fig. 2.

The quantity R_{c_0} was found to be a Langmuir-type function of the initial concentration of crotonic acid. Thus at constant temperature, pressure, volume and amount of catalyst

$$R = \left[\frac{Kac}{1 + bc}\right]\left[\frac{1}{1 + r(c_0 - c)}\right]$$

It was thought of importance to determine whether the rate R should be expressed as $-(dc/dt)$ or as $-(dn/dt)$, where n is the number of moles of crotonic acid at time t. Experiments at constant concentration and experiments with variable volume of ethanol indicated conclusively that the correct expression is $-dn/dt$ (equivalent to $-V\,dc/dt$).

The value of the ratio (Ka/b) determines the maximum value reached by R_{c_0} at high initial concentrations of crotonic acid. This maximum and, therefore, the value of (Ka/b), is determined by the activity of the catalyst. A value of b = 19.2 l./

mole was found to be satisfactory, and was held constant for all catalysts. Ka is thus proportional to the activity of the catalyst.

From the above

$$-\frac{dc}{dt} = \left[\frac{Kac}{V(1 + bc)}\right]\left[\frac{1}{1 + r(c_0 - c)}\right]$$

At a given temperature Ka may be written: $Ka = ka\ f(A)\ f'(P)$. The results have shown that Ka varies linearly with both the amount of catalyst and the hydrogen pressure. Thus $Ka = kaAP$. Appropriate substitution leads to eq. 1, which is integrated to give

$$(1 + rc_0)\ln\left(\frac{c_0}{c}\right) + (c_0 - c)\left[b - r + \frac{br}{2}(c_0 - c)\right] = \left(\frac{kaAP}{V}\right)t \quad (2)$$

The constancy of the ka values reported for each batch of catalyst as each variable is changed is an indication of the validity of eq. 1. Examination of Table I and Table II shows, for eleven experiments with catalyst D covering a 9-fold range of concentrations, that the average value of ka is 0.026 ± 0.001, expressed in the usual units. For six experiments with catalyst C, the average value of ka is

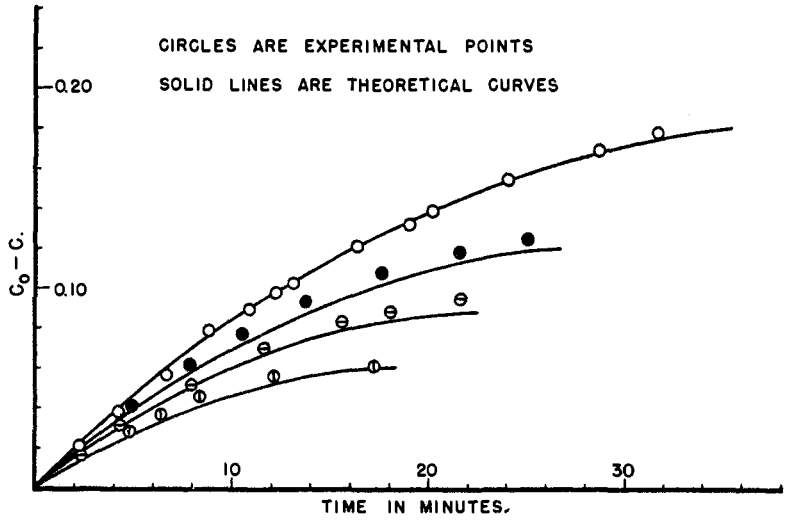

Fig. 5.—The alternate mechanism; data of Fig. 2.

0.041 ± 0.002. The constancy of the ka values reported in Table IV is an indication of the validity of the linear pressure term. The average value for four experiments of 0.028 ± 0.003 for the appropriate set of Table IV is in agreement with the values reported in Tables I and II for other runs with catalyst D. Table IV also shows the constancy of the specific initial slope values, Z, which are related to ka as follows:

$$Z = -\frac{1}{AP}\left(\frac{dc}{dt}\right)_{t=0} = \frac{ka}{V}\frac{c_0}{(1 + bc_0)}$$

It is convenient to consider eq. 2 to be composed of two terms

$$t_A = \frac{V}{kaAP}(c_0 - c)\left[b - r + \frac{br}{2}(c_0 - c)\right], \text{ and}$$

$$t_B = \frac{V}{kaAP}(1 + rc_0)\ln\left(\frac{c_0}{c}\right), \text{ with}$$

$t = t_A + t_B$. In the early stages of the reaction $br/2 \cdot (c_0 - c) << (b - r)$. Then, if c_0 is sufficiently large, $(c_0 - c)\ (b - r)$ is larger than $\ln (c_0/c)$ and the zero-order term dominates. At high extent of reaction the "dominant role" is played by the $\ln (c_0/c)$ term; therefore, near the end of the reaction first-order kinetics are followed.

If c_0 is sufficiently small, $(c_0 - c)$ will be smaller at a given time than $\ln (c_0/c)$, implying that at low initial concentrations the kinetic curve displays nearly first-order kinetics.[19] An extreme case of nearly first order form at low concentration is seen in the work of Vandenheuvel.[11]

Mechanism.—During the course of the hydrogenation, there are at least three species adsorbing on the catalyst surface: hydrogen, crotonic acid and butyric acid. After elimination of several possible mechanisms which do not explain the retardation by product, the following mechanisms are considered: *1. Single class of sites.*—Competitive adsorption of hydrogen, crotonic acid and butyric acid on a single type of surface site. *2. Dual sites.* (a) Crotonic acid adsorbed on sites of type X, hydrogen and butyric acid on sites of type Y. (b) Hydrogen adsorbed on sites of type X, crotonic and butyric acids on sites of type Y. At constant pressure and temperature the single site assumption leads to the following expression for the initial rate

$$R_{c_0} = \frac{Kac_0}{(1 + bc_0)^2}$$

implying that the initial rate goes through a maximum at $c_0 = 1/b$. No maximum was observed at concentrations up to the experimental limit of 3 M. Using the single site assumption the rate equation including the product becomes

$$R = \frac{Kac}{[1 + bc + r(c_0 - c)]^2}$$

It was not possible to fit the data with this rate expression. Thus this mechanism was rejected.

Of the two dual site mechanisms, (2a) leads to eq. 1 if weak adsorption of hydrogen is assumed. The butyric acid molecule is considered to compete with hydrogen for the sites of type Y, thus progressively retarding the reaction.

The alternate dual site mechanism (2b) was found to lead to the equation

$$\frac{Kat}{V} = (1 + rc_0)\ln (c_0/c) + (b - r)(c_0 - c)$$

(19) As a special case, one may consider the situation where $r << b$, *i.e.*, negligible retardation by product. Then

$$t = \frac{V}{kaAP}\left[b(c_0 - c) + \ln\left(\frac{c_0}{c}\right)\right]$$

A set of experiments on the hydrogenation of cyclohexene was performed. The data were accurately fitted by this simple two-parameter equation. This suggests that cyclohexane is negligibly adsorbed on the active centers. Several hydrogenations of maleic acid also were run. Zero-order kinetics were obtained for practically the entire course of the reaction. On the basis of the few curves obtained, the results cannot be quantitatively interpreted. One might suppose, however, that b is quite large and r is small; *i.e.*, the product, succinic acid, is weakly adsorbed. If it is assumed that b is very large, so that $bc >> 1$ for most of the reaction, then $-(dc/dt) = kaAP/bV$, so $t = (bV/kaAP)(c_0 - c)$, in agreement with the observations.

In the early stages the concentration of butyric acid is negligible and the previous evaluation of b is valid. By substituting the data for two points of a single kinetic curve and solving the resulting simultaneous equations, values of r and Ka are obtained.

The solid curves in Fig. 5 are the theoretically calculated curves representing the same data as Fig. 2. It is seen that the data are fairly well fitted, so that this alternate molecular mechanism is also acceptable. The butyric acid is here assumed to compete with crotonic acid for sites of type Y, thus progressively retarding the reaction.

Since the data can be expressed by either of the dual site rate laws, there seems to be no kinetic method of distinguishing between the two mechanisms. Since differential analysis of the data led to convenient evaluation of the constants of eq. 1, this rate law has been used throughout.

One can predict from either mechanism that the catalytic hydrogenation of crotonic acid follows zero-order kinetics initially and terminates by a first-order process. The duration of the zero-order portion of the curve is greater for high initial concentrations of acceptor. Butyric acid inhibits the reaction rate. The acceptable rate laws imply that the catalytic surface consists of two types of active centers, in agreement with the concepts of Balandin.[15]

Acknowledgment.—Thanks are due to Prof. R. L. Burwell, Jr., of Northwestern University for helpful discussions.

CHICAGO 16, ILLINOIS

[CONTRIBUTION NO. 1144 FROM THE STERLING CHEMISTRY LABORATORIES, YALE UNIVERSITY, AND FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY]

The Fractionation of Insulin by Electrophoresis–Convection[1]

BY SERGE N. TIMASHEFF, RAYMOND A. BROWN AND JOHN G. KIRKWOOD

RECEIVED FEBRUARY 4, 1953

An electrophoretic study, carried out in a whole spectrum of buffers, demonstrated that insulin is not a homogeneous protein, but is composed of a principal rapid moving component and a smaller amount of slower electrophoretically broadly spread out material. Fractionation by electrophoresis–convection at *p*H 7.5 resulted in a partial separation of the two components as demonstrated electrophoretically and by activity determinations.

Introduction

Although in the last few years extensive work has been reported on the characterization of insulin, comparatively little literature is available on the problem of insulin homogeneity by physico-chemical criteria.[2-4]

When preliminary electrophoretic analyses, carried out in our laboratories, pointed to the electrophoretic non-homogeneity of insulin, it was decided to make a more complete investigation of this and to attempt the fractionation of this protein by the method of electrophoresis–convection. The results of these studies are reported in this paper.

Experimental

The insulin used was Lilly Zinc-crystalline Insulin, Lot No. 515499 with an activity of 27 units/mg, and also a specially prepared sample of Lilly amorphous insulin No. 200-1B-11J, with an activity of 24 units/mg.

Electrophoresis experiments were performed at 2° in a Klett Tiselius electrophoresis apparatus and also at 0° in a Perkin-Elmer apparatus, Model 38. The electrophoresis–convection runs were carried out in a cold room at 3–4°. The details of construction and operation of the apparatus have been described previously.[5]

In each fractionation run, the insulin was dissolved in the appropriate volume of buffer and dialyzed for 24 hours against the same buffer in the cold room. In the case of zinc-crystalline insulin, in order to remove the zinc, the protein was dissolved first in a *p*H 3.0 HCl solution, the ionic strength of which had been adjusted to 0.1 with NaCl. This solution was then dialyzed for 72 hours against several changes of the HCl and finally brought to the conditions of the experiment prior to fractionation. At the end of each run the top and bottom fractions were removed out of the electrophoresis–convection cell and analyzed electrophoretically.

The samples for activity assay were prepared as follows. The solution was dialyzed against several changes of the *p*H 3.0 HCl solution, then several changes of distilled water and finally lyophilized. The insulin activity determinations were carried out for us at the Lilly Research Laboratories through the courtesy of Dr. E. D. Campbell and Dr. O. K. Behrens.

Results

Electrophoretic Analyses.—The insolubility of insulin in its isoelectric region makes it impossible to carry out electrophoretic analyses in the *p*H zone between 4.4 and 7.0. A number of analyses were carried out, however, under various conditions in the solubility regions on both sides of the zone of insolubility.

In Fig. 1 are shown some typical patterns obtained at various *p*H's. The mobility data are summarized in Table I. From the pictures presented it can be seen that insulin does not migrate electrophoretically in a manner expected of a homogeneous protein, but considerable resolution into components may be observed.

In a *p*H 8.6 barbital buffer ($\Gamma/2 = 0.1$) (Fig. 1a) the pattern shows the presence of a principal component comprising 79% of the total protein with a mobility of -6.74×10^{-5}, and a trailing shoulder with a mean mobility of -5.55×10^{-5}. The component analyses of the rising and descending bound-

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(2) (a) J. L. Hall, *J. Biol. Chem.*, **139**, 175, 671 (1941); (b) E. Volkin, *ibid.*, **175**, 675 (1948).

(3) J. Lens, *Biochim. Biophys. Acta*, **2**, 76 (1948).

(4) E. Fredericq and H. Neurath, THIS JOURNAL, **72**, 2684 (1950).

(5) J. R. Cann, J. G. Kirkwood, R. A. Brown and O. J. Plescia, *ibid.*, **71**, 1603 (1949).