with change in acidity while the 225 mμ peak shifted to longer wave lengths with increasing acidity.

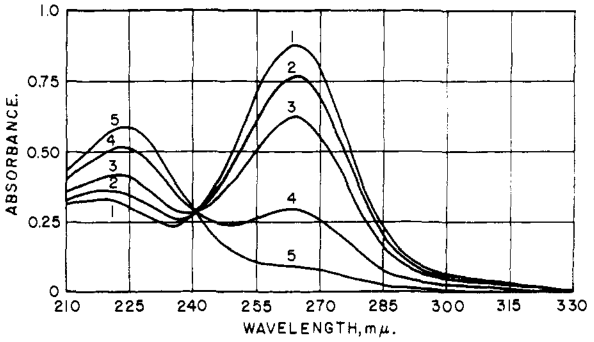

Fig. 1.—Change of absorbance of nitroguanidine with acidity: curve 1, *p*H 7; curve 2, 1.1 *N* HCl; curve 3, 2.07 *N* HCl; curve 4, 4.02 *N* HCl; curve 5, 6.10 *N* HCl. Cary Model 11MS recording spectrophotometer; 1-cm. matched silica cells; temperature 25°.

When the peak absorbance values were plotted against hydrogen ion concentration (*p*H), there was a sharp break in the curve. In the usual application of this method,[2] $pK_b = pOH$ at the mid-point of the break in the curve. For this experiment conducted in hydrochloric acid solutions, pK_b = 14.45 and 14.55 when conducted in sulfuric acid solutions. In these runs there was complete return of the absorption maximum at 265 mμ when the individual strong acid solutions were brought back to *p*H of 7. This indicates complete recovery of the nitroguanidine and that negligible decomposition occurred while the solutions were strongly acidic.

Similar experiments were made with nitroaminoguanidine. In this case the minor peak at about 225 mμ increased very little in absorbance and the decrease in absorbance at 267 mμ was much less than observed for nitroguanidine with increasing acidity. The plot of absorbance *versus* *p*H gave a much smaller break. The $pOH = pK_b$ for this case was estimated at approximately 15.0.

Previous work has shown that nitroaminoguanidine was the stronger acid of the two compounds, pK_a = 10.60 for nitroaminoguanidine and 12.20 for nitroguanidine. This difference in acid strength and similarity in base strength appears to be consistent with the structure of the two compounds.

(2) J. E. De Vries and E. St. C. Gantz, This Journal, **76**, 1008 (1954).

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The Evaluation of the Initial Velocities of Enzyme-catalyzed Reactions that Are Competitively Inhibited by their Reaction Products[1]

By William E. M. Lands[2] and Carl Niemann[3]

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A number of enzyme-catalyzed reactions that are competitively inhibited by their reaction products may be represented by equations 1, 2 and 3 and under certain circumstances, *i.e.*, when $-d[ES]/dt \doteq 0$, $[S_f] \doteq [S]$, $[P_{1f}] \doteq [P_1]$ and $[P_{2f}] \doteq [P_2]$,

$$E_f + S_f \underset{k_2}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_3} E_f + P_{1f} + P_{2f} \ldots \quad (1)$$

$$E_f + P_{1f} \underset{k_5}{\overset{k_4}{\rightleftarrows}} EP_1 \quad (2)$$

$$E_f + P_{2f} \underset{k_7}{\overset{k_6}{\rightleftarrows}} EP_2 \ldots \quad (3)$$

such reactions may be described throughout their course in terms of equation 4

$$-d[S]/dt = \{[E][S](k_3K_P/(K_P - K_S))\}/\{(K_S(K_P + [S]))/(K_P - K_S) + [S]\} \quad (4)$$

where $K_S = (k_2 + k_3)/k_1$, $K_P = 1\Big/\sum_{j=1}^{n} 1/K_{P_j}$, $K_{P_1} = k_5/k_4$ and $K_{P_2} = k_7/k_6$. While it has been shown[4-7] that the initial velocities of the above reactions may be evaluated by graphical procedures based upon definite or indefinite integration of equation 4 and subsequent rearrangement of the integrated equations it also has been emphasized[7] that such procedures are applicable only when the reaction in question has been allowed to proceed to an extent compatible with the eventual evaluation of K_P. Since the attainment of this goal will in part be determined not only by the absolute magnitude of K_P but also by the relative magnitudes of K_P and K_S, it will be appreciated that experiments conducted in the absence of prior knowledge of the values of K_P and K_S may frequently yield data that are incapable of precise evaluation by any one of the above graphical procedures. However, since such data can be used in conjunction with any one of the above graphical procedures to determine approximate values of K_P and K_S we believe it worth while to describe an evaluation procedure that can be used to determine initial velocities in cases of this kind as well as in those in which an added competitive inhibitor is also present.

An enzyme-catalyzed reaction that is competitively inhibited by its reaction products and by an added competitive inhibitor may be represented by equations 1, 2, 3 and 5 and under certain circumstances such a reaction may be described

$$E_f + I_f \underset{k_9}{\overset{k_8}{\rightleftarrows}} EI \quad (5)$$

throughout its course by equation 6 where $K_I = k_9/k_8$.

$$k_3[E]t = K_S(1 + [S]_0/K_P + [I]/K_I) \ln([S]_0/[S]_t) + (1 - K_S/K_P)([S]_0 - [S]_t) \quad (6)$$

When $[I] = 0$ and $K_P >> K_S$, equation 6 is approximated by equation 7. It has been shown

$$k_3[E]t = K_S \ln([S]_0/[S]_t) + ([S]_0 - [S]_t) \quad (7)$$

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(1) Supported in part by a grant from Eli Lilly and Co.

(2) National Science Foundation Postdoctoral Fellow.

(3) To whom inquiries regarding this article should be sent.

(4) R. J. Foster and C. Niemann, *Proc. Natl. Acad. Sci.*, **39**, 999 (1953).

(5) T. M. Applewhite and C. Niemann, This Journal, **77**, 4923 (1955).

(6) R. R. Jennings and C. Niemann, *ibid.*, **77**, 5432 (1955).

(7) K. A. Booman and C. Niemann, *ibid.*, **77**, 5733 (1955).

previously[8] that for a reaction described by equation 7 the initial velocities will be determined by the parameters $([S]_0 - [S]_t)$ and t_0' where $t_0'/t = f_0$ is given by equation 8, in which the reaction parameter $P = ([S]_0 \ln ([S]_0/[S]_t))/([S]_0 - [S]_t)$,

$$f_0 = ((K_S/[S]_0) + 1)/(P(K_S/[S]_0) + 1) \qquad (8)$$

or alternatively by the parameters $[S]_0 \ln ([S]_0/[S]_t)$ and t_1' where $t_1'/t = f_1$ is given by equation 9

$$f_1 = ((K_S/[S]_0) + 1)/((K_S/[S]_0) + (1/P)) \qquad (9)$$

When $[I] = 0$ equation 6 is reduced to equation 10 and for this case it can be shown that the initial

$$k_3[E]t = K_S(1 + [S]_0/K_P) \ln ([S]_0/[S]_t) + (1 - K_S/K_P)([S]_0 - [S]_t) \qquad (10)$$

velocities will be determined by the same parameters as those noted immediately above except that the factors f_0 and f_1 are now given by equations

$$f_0 = \{((K_S/[S]_0) + 1)/(1 - (K_S/K_P))\}/\{P\{((K_S/[S]_0) + (K_S/K_P))/(1 - (K_S/K_P))\} + 1\} \qquad (11)$$

$$f_1 = \{((K_S/[S]_0) + 1)/(1 - (K_S/K_P))\}/\{((K_S/[S]_0) + (K_S/K_P))/(1 - (K_S/K_P)) + 1/P\} \qquad (12)$$

11 and 12. It will be seen from equations 11 and 12 that when $K_P = K_S$ equation 11 is reduced to $f_0 = 1/P$ and equation 12 is reduced to $f_1 = 1$. Thus, for this particular situation, *i.e.*, where $v_0 = [S]_0 \ln ([S]_0/[S]_t)/t_1' = [S]_0 \ln (([S]_0 \ln ([S]_0/[S]_t))/t$, it is particularly important to evaluate the relation $t_0' = f_0 t$ in order to distinguish a reaction system represented by equations 1, 2 and 3 where $K_P = K_S$, from the situation represented by equation 1 where $[S]_0$ is very small relative to K_S since both of these systems will appear to be pseudo first order with respect to the specific substrate.[9]

For a reaction system described by equation 6 the initial velocities will be determined by the same parameters as before except that in this instance the factors f_0 and f_1 are given by equations 13 and 14. As in the case considered immediately

$$f_0 = \{((K_S/[S]_0)(1 + ([I]/K_I)) + 1)/(1 - (K_S/K_P))\}/\{P\}((K_S/[S]_0)(1 + ([I]/K_I)) + (K_S/K_P))/(1 - (K_S/K_P))\} + 1\} \qquad (13)$$

$$f_1 = \{((K_S/[S]_0)(1 + ([I]/K_I)) + 1)/(1 - K_S/K_P))\}/\{((K_S/[S]_0)(1 + ([I]/K_I)) + (K_S/K_P))/(1 - (K_S/K_P)) + 1/P\} \qquad (14)$$

above the reaction system may again appear to be pseudo first order with respect to the specific substrate when $K_P = K_S$ because again $f_1 = 1$ and $v_0 = [S]_0 \ln ([S]_0/[S]_t)/t' = [S]_0 \ln ([S]_0/[S]_t)/t$.[10]

In order to distinguish between the three reaction systems being considered and for simplification we shall introduce the substitutions which are summarized in Table I. With the quantities a and b defined as in Table I we may now replace equations 8, 11 and 13 by equation 15 and equations 9, 12 and 14 by equation 16.

$$f_0 = 1/(P)((a + b)/a) - (b/a) \qquad (15)$$

$$f_1 = 1/((a + b)/a) - (b/a)(1/P) \qquad (16)$$

TABLE I

DEFINITIONS OF PARAMETERS a AND b

Equations	a	b
8, 9	$((K_S/[S]_0) + 1)$	-1
11, 12	$((K_S/[S]_0) + 1)$	$((K_S/K_P) - 1)$
13, 14	$((K_S/[S]_0)(1 + ([I]/K_I)) + 1$	$((K_S/K_P) - 1)$

In an earlier communication[8] a procedure was described for the evaluation of f_0 and f_1 for the case described by equation 7, *i.e.*, where f_0 and f_1 are given by equations 8 and 9. While this procedure was satisfactory for the application originally intended[8] it is not suitable for the other cases considered in this study. Consequently attention has been directed to the development of a more general procedure for the evaluation of f_0 and f_1.

A comparison of equations 15 and 16 leads to the relationship between f_0 and f_1 which is given by equations 17 and 18. Thus, the evaluation of

$$f_1 = a/((a + b) - (b/P)) = P/(P)((a + b)/a) - (b/a) \qquad (17)$$

$$f_1 = Pf_0 \qquad (18)$$

either f_0 or f_1 will lead to the evaluation of the other through the use of the relationship given by equation 18. In this communication we shall limit our attention to a procedure for the evaluation of f_0 because of the relative ease of handling the zero-order case. However, it should be appreciated that a similar procedure may be developed for the evaluation of f_1.

Equation 15 may be rearranged to give equation 19 which can be transformed into its determinant,

$$1/f_0 - P((a + b)/a) + (b/a) = 0 \qquad (19)$$

the elements in row two and columns one and two of this determinant multiplied by the quantity $(a/b)/(a/b)$ to give equation 20, which in turn may be used to construct a nomogram which

$$\begin{vmatrix} 0 & \mu_1(1/f_0) & 1 \\ \dfrac{-\delta_3(1 + (b/a))}{(\mu_3/\mu_1) - (1 + (b/a))} & \dfrac{-\mu_3(b/a)}{(\mu_3/\mu_1) - (1 + (b/a))} & 1 \\ \delta_3 & \mu_3 P & 1 \end{vmatrix} = 0 \qquad (20)$$

will describe the interrelationships of the variables of interest.[11] Since experimental conditions may vary greatly it may be desirable to select different scale factors, *i.e.*, values of μ_1, μ_3 and δ_3 for markedly different systems. While these scale factors also may be selected to give nomograms of any size and shape it has been found that for many of the situations encountered in these laboratories the nomogram, *cf.*, Fig. 1, based upon the constructional determinant given by equation 21 is satisfactory for the evaluation of f_0 in those cases where values of P are limited to those corresponding to

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(8) R. R. Jennings and C. Niemann, THIS JOURNAL, **75**, 4687 (1953).

(9) We are of the opinion that situations where $K_P = K_S$ within the limits of experimental error are relatively common and for this reason we believe that caution should be exercised in assuming that enzyme-catalyzed reactions which appear to be first order with respect to the specific substrate are necessarily due to a reaction system which can be represented by equation 1 where $[S]_0$ is very small relative to K_S.

(10) It will be seen that in investigations directed toward the determination of the K_I values of competitive inhibitors an operational advantage is gained if such inhibitors can be evaluated in systems where $K_P = K_S$. Therefore, the specific substrates used in such studies should be selected with this criterion in mind.

(11) H. J. Allcock and J. R. Jones, "The Nomogram," I. Pitman and Sons, Ltd., London, 1932.

$$\begin{vmatrix} 0 & 375/f_0 & 1 \\ \dfrac{-50((a/b)+1)}{0.333(a/b)-1} & \dfrac{-375(a/b)}{0.333(a/b)-1} & 1 \\ 50 & 500P & 1 \end{vmatrix} = 0 \qquad (21)$$

ca. 15% reaction. The nomogram which is presented in Fig. 1 is given only to illustrate the general nature of the nomograms which may be developed from equation 20.

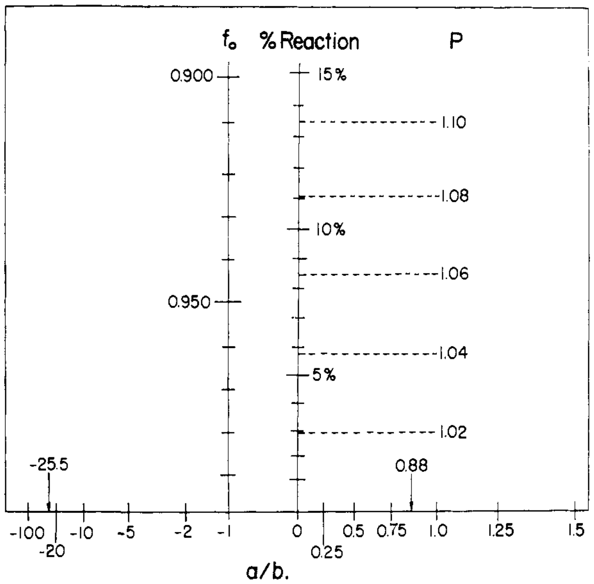

Fig. 1.—Nomogram for the evaluation of f_0.

In Fig. 1 the reaction parameter P is given as such and also in terms of percentage reaction. Since x, the percentage reaction, is equal to the quantity $100(([S]_0 - [S]_t)/[S]_0)$ it follows that $P = \{\ln(100/(100 - x))\}/(x/100)$. For the construction of the dual scales involving the reaction parameters values of P, or of x, are most readily obtained from a plot such as that given in Fig. 2.

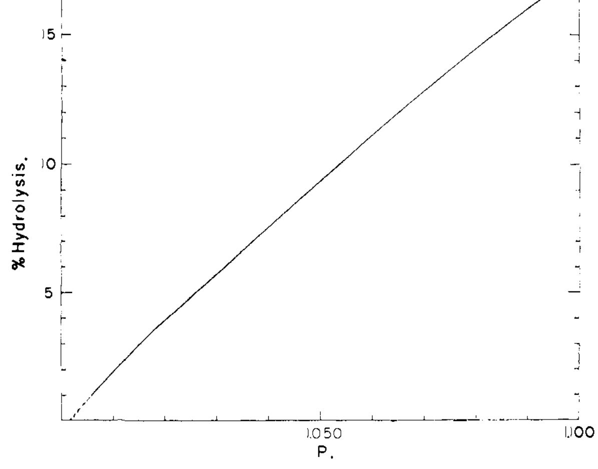

Fig. 2.—Plot of the reaction parameter $P = \{\ln(100/(100 - x))\}/(x/100)$ *vs.* x = % reaction, *i.e.*, hydrolysis.

The application of the procedures described in this communication may be illustrated by the following example. An α-chymotrypsin-catalyzed hydrolysis of acetyl-L-tyrosinhydrazide in aqueous solutions at 25° and *p*H 7.95 and 0.02 *M* in the THAM[12] component of a THAM–HCl buffer under conditions where [E] = 0.145 mg. protein-nitrogen/ml. and $[S]_0 = 2.454 \times 10^{-3}$ *M* was followed by determining the amount of liberated hydrazine by its subsequent reaction with *p*-dimethylaminobenzaldehyde to give the colored bis-*p*-dimethylaminobenzalazine.[13] The relationship observed between the time t in minutes and percentage hydrolysis is given in Table II.

Initially it was assumed that the reaction in question could be represented by equation 1, that its rate equation was given by equation 7, that the factor f_0 was described by equation 8 and that $K_S = 60 \times 10^{-3}$ *M*. For this situation it is seen from Table I that the quantity $a/b = -((60/2.454) + 1) = -25.5$. With this value for a/b values of the factor f_0 were obtained from the nomogram and these latter values were used to compute the corresponding values of t_0', *cf.*, Table II. A plot of $([S]_0 - [S]_t)$ *vs.* t_0' was then constructed and from this plot, which was linear within the limits of experimental error, a value of $v_0 = 11.6 \times 10^{-6}$ *M*/min. was obtained.

TABLE II

α-CHYMOTRYPSIN-CATALYZED HYDROLYSIS OF ACETYL-L-TYROSINHYDRAZIDE[a]

t (min.)	% Hydrol.[b]	f_0 Eq. 8	f_0 Eq. 11	t_0' Eq. 8	t_0' Eq. 11
0	0.0	1.000	1.000	0.0	0.0
3	1.39	0.993	0.985	3.0	3.0
6	2.79	.989	.970	5.9	5.8
9	4.25	.980	.952	8.8	8.6
12	5.57	.973	.939	11.7	11.3
15	6.85	.968	.925	14.5	13.9
18	8.22	.960	.910	17.3	16.4
21	9.34	.955	.899	20.1	18.9
24	10.73	.948	.885	22.8	21.2

[a] In aqueous solutions at 25° and *p*H 7.95 and 0.02 *M* in the THAM component of a THAM–HCl buffer where [E] = 0.145 mg. protein–nitrogen/ml. and $[S]_0 = 2.454 \times 10^{-3}$ *M*, [b] *i.e.*, $100\,([S]_0 - [S]_t)/[S]_0$.

Recognizing that evidence for the existence of a reaction represented by equations 1, 2 and 3 can best be obtained by a plot based upon equation 10^{4-7} a plot of $([S]_0 - [S]_t)/t$ *vs.* $(\ln([S]_0/[S]_t))/t$ was constructed and while this plot was of such a nature that it could not be used for the evaluation of v_0 it was sufficiently informative to reveal that $K_P < K_S$ and that K_P could be assumed to have a value of 2×10^{-3} *M*. Therefore, it was decided to evaluate v_0 by the procedure described in this communication using a value of $K_S = 60 \times 10^{-3}$ *M* and a value of $K_P = 2 \times 10^{-3}$ *M*.

For a reaction described by equation 10 the factor f_0 is given by equation 11 and it is seen from Table I that for the case at hand the quantity $a/b = ((60/2.454) + 1)/((60/2) - 1) = 0.88$. With this value of a/b values of the factor f_0 were obtained from the nomogram and these latter values were used to calculate the corresponding values of t_0', *cf.*, Table II. As before a plot of

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(12) Tris-(hydroxymethyl)-aminomethane.

(13) R. Lutwack, Ph.D. Thesis, Calif. Inst. Tech., Pasadena, 1955.

($[S]_0 - [S]_t$) *vs.* t_0' was constructed and from this plot, which was linear within the limits of experimental error, a value of $v_0 = 12.2 \times 10^{-6}$ M/min. was obtained.

In addition to the application described above the nomogram developed in this communication has proved to be useful in predicting the effect of changes in values of K_S, K_P and K_I and of $[S]_0$ and $[I]$ upon the initial velocities and thus has provided a rapid and convenient way to anticipate the consequences of a particular experiment and whether such an experiment will be definitive.

The authors wish to express their indebtedness to Mr. Charles Goebel for his assistance in the construction of the nomogram described in this communication.

CONTRIBUTION NO. 2022 FROM THE
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The Vapor Pressure of Bromine from 24 to 116°[1]

BY JACK FISCHER AND JAMES BINGLE

RECEIVED AUGUST 31, 1955

The vapor pressure of bromine was determined from 24 to 116° in a nickel and Monel apparatus in which the vapor was cycled through the liquid with the use of a magnetically operated pump.

The apparatus employed in this investigation has been described in another publication.[2] Reagent grade bromine was purified by distilling off a large portion, about one-quarter of the original volume, at room temperature, to remove any chlorine present. The remainder was passed over phosphorus pentoxide in order to remove traces of water before adding it to the system. The sample to be used was condensed in a fluorothene trap cooled with liquid nitrogen. Approximately 15- to 20-ml. portions were collected in the trap at a time. Each portion was frozen, evacuated and thawed several times to remove non-condensable gases. A 3- to 4-ml. portion of the bromine in the trap was distilled off to remove any further impurities more volatile than bromine. The residual amount of bromine was then distilled into the equilibrium cell of the apparatus. The process was repeated until 80–90 ml. of bromine had been collected in the equilibrium cell.

Vapor pressure measurements were made when a constant value for the vapor pressure at 75° was obtained after removing successive fractions of bromine from the system. The fact that the reduction of the volume of bromine to half its value produced no change in vapor pressure at 75° is evidence of its purity.

The vapor pressure of bromine from 24 to 116° is expressed by the equation

$$\log p = -\frac{2199.0}{T} - 4.150 \log T + 19.9618 \quad (1)$$

where p = pressure in millimeters, T = temperature °K. The data are shown in Table I and the log of the pressure in millimeters is plotted against the temperature in Fig. 1. The pressures obtained by Ramsay and Young[3] and those obtained by Scheffer and Voogd[4] are plotted in Fig. 2 together with our values over a representative range. Our values agree with those of Ramsay and Young within experimental error and are only slightly lower than those of Scheffer and Voogd. We have probably achieved a better removal of more volatile impurities. Values for the vapor

TABLE I

VAPOR PRESSURE OF BROMINE, 24 TO 116°

Temp., °C.	Observed pressure mm.	% dev. Obsn. *vs.* calcn. eq. 1	Temp., °C.	Observed pressure mm.	% dev. Obsn. *vs.* calcn. eq. 1
24.48	202	−0.5	70.16	1083	+0.3
26.66	223	.0	74.87	1246	− .1
32.37	283	.0	79.80	1439	− .1
43.44	437	+ .2	85.89	1716	+ .2
49.67	548	+ .2	93.91	2129	+ .2
55.00	662	+ .5	100.86	2527	− .5
60.32	787	− .3	107.04	2957	.0
64.06	892	+ .1	111.21	3268	+ .1
66.90	977	+ .1	116.19	3662	− .1
				Av.	±0.19

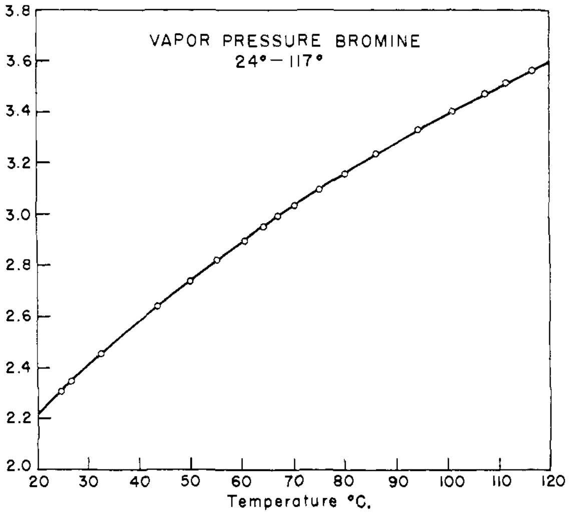

Fig. 1.—$\log_{10} p$ (in mm.) *vs.* temperature, °C.

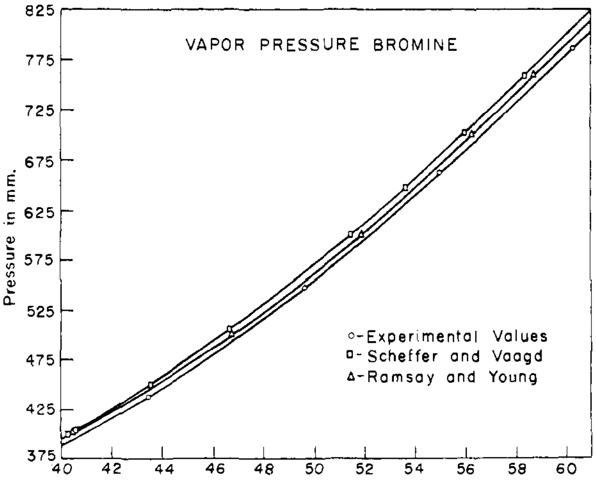

Fig. 2.—Vapor pressure (mm.) of bromine *vs.* temperature, °C.

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[1] Work performed under the auspices of the U. S. Atomic Energy Commission.

[2] J. Fischer, J. Bingle and R. C. Vogel, THIS JOURNAL, in press.

[3] J. Ramsay and J. Young, *J. Chem. Soc.*, **49**, 453 (1886).

[4] F. E. C. Scheffer and M. Voogd, *Rec. trav. chim.*, **45**, 214 (1926).