bamic acid) is required in the reaction, experiments were performed by direct reaction of hydrogen sulfide and 2-methyl-2-isothiocyano-4-pentanone. Under the experimental conditions employed, which included reactions at both atmospheric and super atmospheric pressure, none of the desired product was obtained.

4,4,6-Trimethyl-4H-1,3-thiazine-2-thiol (II).—A sample of I (prepared according to method B) was heated with sulfuric acid to convert it to II, as previously described. Recrystallization from carbon tetrachloride gave a product melting at 120–121°. The melting point of a mixture of this product with II derived from I prepared according to method A (m.p. 120.5–121.5°) was unchanged.

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The Evaluation of the Kinetic Constants of Enzyme-catalyzed Reactions by Procedures Based upon Integrated Rate Equations

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It is generally recognized that the course of an enzyme-catalyzed reaction that can be represented by equation 1 can, under certain circumstances, be

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

described either in terms of a differential rate equation, *i.e.*, equation 2, or an integrated rate equation, *i.e.*, equation 3. While it is known that the con-

$$-\mathrm{d[S]}/\mathrm{d}t = v_0 = k_3[\mathrm{E}][\mathrm{S}]/(K_\mathrm{S} + [\mathrm{S}]) \quad (2)$$

$$k_3[\mathrm{E}]t = K_\mathrm{S} \ln ([\mathrm{S}]_0/[\mathrm{S}]_\mathrm{t}) + ([\mathrm{S}]_0 - [\mathrm{S}]_\mathrm{t}) \quad (3)$$

stants $K_\mathrm{S} = (k_2 + k_3)/k_1$ and k_3 may be evaluated by any one of three graphical procedures based upon equation 2[2,3] it apparently has not been appreciated that in addition to the procedure described by Walker and Schmidt[4] there are two other graphical procedures that can be derived from equation 3. The characteristics of these two new procedures as well as those of the four described previously[2–4] are summarized in Table I.

We do not intend at this time to discuss the relative merits of each of the plots given in Table I other than to point out that all of the plots based upon equation 2 require a separate estimation of the initial velocities. Although the initial velocities can be evaluated in an objective manner[5] it has been our experience that any procedure which requires such an evaluation is, in general, less convenient than one which does not. A far more serious consideration is that for most enzyme-catalyzed reactions equations 2 and 3 may be valid only for the initial stages of these reactions and while useful information can be obtained by observation of this phase of a given reaction it is clear that procedures which are based upon a rate equation which describes the reaction throughout its entire course are the more desirable.

There are a relatively large number of enzyme catalyzed reactions which can be represented by equations 1, 4 and 5, and under certain circumstances

$$\mathrm{E_f} + \mathrm{P_{1f}} \underset{k_5}{\overset{k_4}{\rightleftarrows}} \mathrm{EP_1} \quad (4)$$

$$\mathrm{E_f} + \mathrm{P_{2f}} \underset{k_7}{\overset{k_6}{\rightleftarrows}} \mathrm{EP_2} \ldots \quad (5)$$

these reactions can be described over their entire course in terms of an integrated rate equation, *i.e.*, equation 6, in which K_{P_1}, K_{P_2}, etc., are the respective dissociation constants of the complexes formed by the reversible interaction of the enzyme with the reaction products.

$$k_3[\mathrm{E}]t = K_\mathrm{S} \left(1 + [\mathrm{S}]_0 \sum_{j=1}^{n} 1/K_{\mathrm{P}_j}\right) \ln ([\mathrm{S}]_0/[\mathrm{S}]_\mathrm{t}) + \left(1 - K_\mathrm{S} \sum_{j=1}^{n} 1/K_{\mathrm{P}_j}\right)([\mathrm{S}]_0 - [\mathrm{S}]_\mathrm{t}) \quad (6)$$

It has been shown[6,7] that when $\sum_{j=1}^{n} 1/K_{\mathrm{P}j} = 1/K_\mathrm{P}$ equation 6 may be rearranged to the slope–intercept form given in equation 7 and that a plot of

$$([\mathrm{S}]_0 - [\mathrm{S}]_\mathrm{t})/t = \{k_3[\mathrm{E}]K_\mathrm{P}/(K_\mathrm{P} - K_\mathrm{S})\} - \{K_\mathrm{S}(K_\mathrm{P} + [\mathrm{S}]_0)(\ln ([\mathrm{S}]_0/[\mathrm{S}]_\mathrm{t}))/t(K_\mathrm{P} - K_\mathrm{S})\} \quad (7)$$

$([\mathrm{S}]_0 - [\mathrm{S}]_\mathrm{t})/t$ *vs.* $(\ln ([\mathrm{S}]_0/[\mathrm{S}]_\mathrm{t}))/t$ will give a family of lines of slope $-K_\mathrm{S}(K_\mathrm{P} + [\mathrm{S}]_0)/(K_\mathrm{P} - K_\mathrm{S})$ and a common ordinate intercept of $k_3[\mathrm{E}]/(1 - K_\mathrm{S}/K_\mathrm{P})$[8] for various values of $[\mathrm{S}]_0$. It also has been shown[6,7] that the points of intersection of the lines of slope $-K_\mathrm{S}(K_\mathrm{P} + [\mathrm{S}]_0)/(K_\mathrm{P} - K_\mathrm{S})$ for particular values of $[\mathrm{S}]_0$ with lines of slope $[\mathrm{S}]_0$ which are drawn through the origin are the points corresponding to $t = 0$ and that a line drawn through the points corresponding to $t = 0$ will have a slope of $-K_\mathrm{S}$, an ordinate intercept of $k_3[\mathrm{E}]$ and an abscissa intercept of $k_3[\mathrm{E}]/K_\mathrm{S}$. While the above plot employs the same coördinates as the plot of Walker and Schmidt[4] and, in some respects, is similar to the plot of Eadie,[3] *cf.*, Table I, it is superior to both of these latter plots in that the quantity K_P may be simultaneously evaluated under conditions which do not require the separate addition of the reaction products and the initial velocities, which are defined in the above plot in terms of the ordinate $([\mathrm{S}]_0 - [\mathrm{S}]_\mathrm{t})/t$ for the condition $t = 0$, need not be separately evaluated. Furthermore, in the plot based upon equation 7, all of the experimental data are presented and evaluated in a single plot and it is immediately apparent from the plot whether K_P is less than, equal to, or greater than K_S and whether K_P is independent of the concentration of $[\mathrm{S}]_0$.

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

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(8) It is evident from this relation that when $K_\mathrm{P} > K_\mathrm{S}$ the ordinate intercept will be positive, when $K_\mathrm{P} = K_\mathrm{S}$ the ordinate intercept will be indeterminate, since the lines of slope $-K_\mathrm{S}(K_\mathrm{P} + [\mathrm{S}]_0)/(K_\mathrm{P} - K_\mathrm{S})$ will parallel the ordinate, and when $K_\mathrm{P} < K_\mathrm{S}$ the ordinate intercept will be negative.

Equation 7 may be rearranged to give equation 8 and it follows that in a plot of $t/([S]_0 - [S]_t)$ *vs.* $(\ln([S]_0/[S]_t))/([S]_0 - [S]_t)$ there will be obtained for various values of $[S]_0$ a family of lines of slope $K_S(K_P + [S]_0)/k_3[E]K_P$ with a common ordinate intercept of $(1 - K_S/K_P)/k_3[E]$.[9] For each of the lines of slope $K_S(K_P + [S]_0)/k_3[E]K_P$ there is a point corresponding to $t = 0$ and as before[7] this point may be located by examination of the limits of the two parameters $t/([S]_0 - [S]_t)$ and $(\ln([S]_0/[S]_t))/([S]_0 - [S]_t)$ as $t \rightarrow 0$. Since the limit of $t/([S]_0 - [S]_t)$ as $t \rightarrow 0$ is $1/-d[S]/dt = 1/v_0$ and that of $(\ln([S]_0/[S]_t))/([S]_0 - [S]_t)$ as $t \rightarrow 0$ is $1/[S]_0$ it is evident that the points at which the lines of slope $K_S(K_P + [S]_0)/k_3[E]\ K_P$ possess abscissa values equal to $1/[S]_0$ will be the points where $t = 0$. Therefore, as the parameters of the points corresponding to $t = 0$ for various values of $[S]_0$ are, respectively, $1/-d[S]/dt = 1/v_0$ for the ordinate and $1/[S]_0$ for the abscissa it follows[2] that a line drawn through these points will have a slope of $K_S/k_3[E]$ and an ordinate intercept of $1/k_3[E]$. It will be recognized that this plot is similar to the first of the two plots proposed by Lineweaver and Burk,[2] *cf.*, Table I, but that in the present case the initial velocities need not be separately evaluated since they are given, in the form of their reciprocals, in terms of the ordinate $t/([S]_0 - [S]_t)$ for the condition that $t = 0$. Furthermore, K_P can be simultaneously determined either from the intercept $(1 - K_S/K_P)/k_3[E]$ or the slope $K_S(K_P + [S]_0)/k_3[E]K_P$.

$$t/([S]_0 - [S]_t) = (K_P - K_S)/k_3[E]K_P + \{K_S(K_P + [S]_0)/k_3[E]K_P\}\{(\ln([S]_0/[S]_t))/([S]_0 - [S]_t)\} \quad (8)$$

TABLE I

GRAPHICAL PROCEDURES FOR THE EVALUATION OF K_S AND k_3

Ref.	Ordinate	Abscissa	Ordinate intercept	Slope
2	$1/v_0$	$1/[S]_0$	$1/k_3[E]$	$K_S/k_3[E]$
..	$t/([S]_0 - [S]_t)$	$(\ln([S]_0/[S]_t)/([S]_0 - [S]_t)$		
2	$[S]_0/v_0$	$[S]_0$	$K_S/k_3[E]^a$	$1/k_3[E]$
..	$t/(\ln([S]_0/[S]_t))$	$([S]_0 - [S]_t)/(\ln([S]_0/[S]_t))$		
3	v_0	$v_0/[S]_0$	$k_3[E]^b$	$-K_S$
4	$([S]_0 - [S]_t)/t$	$(\ln([S]_0/[S]_t))/t$		

[a] Abscissa intercept $= -K_S$. [b] Abscissa intercept $= k_3[E]/K_S$.

Equation 7 may also be rearranged to give equation 9 and for this situation a plot of $t/(\ln([S]_0/[S]_t))$ *vs.* $([S]_0 - [S]_t)/(\ln([S]_0/[S]_t))$ will give, for various values of $[S]_0$, a series of parallel lines of slope $(K_P - K_S)/k_3[E]K_P$.[10] It can be shown that the points corresponding to $t = 0$ for the lines of slope $(K_P - K_S)/k_3[E]K_P$ are the points where the lines of slope $(K_P - K_S)/k_3[E]K_P$ possess abscissa values of $[S]_0$ and that a line drawn through these points, whose coördinates are $[S]_0/-d[S]/dt = [S]_0/v_0$ and $[S]_0$ when $t = 0$, will have a slope of $1/k_3[E]$, an ordinate intercept of $K_S/k_3[E]$ and an abscissa intercept of $-K_S$. It will be seen that this plot is similar to the second plot of Lineweaver and Burk,[2] *cf.*, Table I, but is superior to this plot in that for $t = 0$ the ordinate $t/(\ln([S]_0/[S]_t))$ describes the initial velocities in terms of $[S]_0/v_0$. Also K_P may be simultaneously evaluated by equating the observed slope of the parallel lines to the quantity $(K_P - K_S)/k_3[E]K_P$.

$$t/(\ln([S]_0/[S]_t)) = K_S(K_P + [S]_0)/k_3[E]K_P + \{(K_P - K_S)/k_3[E]K_P\}\{([S]_0 - [S]_t)/(\ln([S]_0/[S]_t))\} \quad (9)$$

While it appears that all three of the graphical procedures based upon equation 6 will be found to be superior to those based upon equations 2 and 3, because it is very probable that a large number of enzyme-catalyzed reactions are competitively inhibited by one or more of their reaction products, it is difficult to assert that any one of the procedures which are based upon equation 6 is superior to the other two for all cases that may be encountered. Therefore, for any particular situation it is suggested that all three be given consideration. In conclusion we wish to note that the procedures described in this communication are capable of extension to systems more complicated than those represented by equations 1, 4 and 5. However, we wish to defer discussion of such systems at this time.

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[9] It will be noted that this intercept is the reciprocal of that obtained in the case of a $([S]_0 - [S]_t)/t$ *vs.* $(\ln([S]_0/[S]_t))/t$ plot.

[10] It is seen from this relation that when $K_P > K_S$ the slope will be positive, when $K_P = K_S$ the lines will parallel the abscissa and when $K_P < K_S$ the slope will be negative.

Nitration in the Presence of Polyphosphoric Acid

BY J. P. KISPERSKY AND K. KLAGER

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Steinkopf and Supan previously reported that the nitration of the alkylmalonates required the use of fuming nitric acid–acetic anhydride mixture at 40 to 50°.[1] The authors cautioned against allowing the temperature of the reaction to rise over 50° presumably because an uncontrollable reaction sets in due to the exothermic nature of the nitration. It has been found that polyphosphoric acid may replace the acetic anhydride in the nitration mixture giving good yields of alkylnitromalonates and reducing the potential hazard of the reaction medium.

The compounds thus prepared are listed in Table I along with the yield, b.p., refractive index and analysis. On the basis of the data presented it is evident that branching on the carbon atom attached to the malonic ester moiety reduces the yield on nitration.

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Experimental

Inasmuch as the same experimental procedure was used in the preparation of all of the compounds listed in Table I a general method will be described.

Fifty grams of technical 100% nitric acid was added to 80 g. of polyphosphoric acid[2] contained in a 200-ml. three-necked flask equipped with stirrer, thermometer and dropping funnel. The mixture was heated to 60° and, when homogeneous, 0.14 mole of diethyl alkylmalonate was added dropwise over a period of 15 to 30 minutes. After a small quantity of ester had been added the mixture required slight cooling to maintain a temperature of 60°. After the addition of the ester the reaction was stirred for one

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[1] W. Steinkopf and A. Supan, *Ber.*, **43**, 3239 (1910).

[2] Obtained from the Victor Chemical Works, Chicago 4, Illinois.