forms. Further evidence for the correct assignment of configuration to *threo-* and *erythro-*DOPS has recently been presented by M. Winitz, S. M. Birnbaum and J. P. Greenstein (THIS JOURNAL, **77**, 3106 (1955)).

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Synthesis of Thiazinethiols

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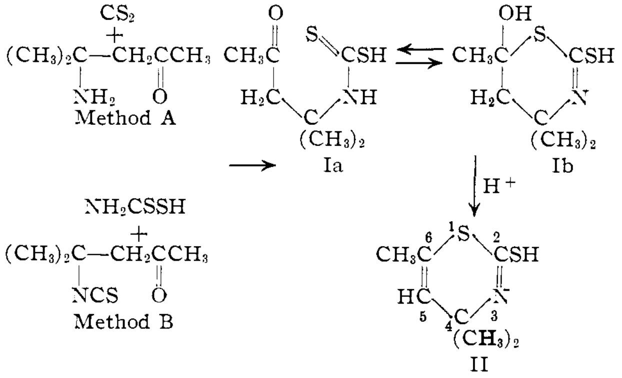
In a previous paper,[1] the synthesis of several alkyl substituted 6H-1,3-thiazine-2-thiols was described. These compounds were prepared by treating α,β-unsaturated ketones with dithiocarbamic acid to form stable intermediates which cyclized to thiazines on treatment with acids. Further investigation has resulted in the discovery of two new synthetic methods for the closely related 4H-1,3-thiazines.

In the first of these methods (method A), diacetoneamine (4-amino-4-methyl-2-pentanone) is treated with carbon bisulfide to give an intermediate, 2-methyl-4-oxopentyldithiocarbamic acid (I).[2] By treatment with sulfuric acid or acetic anhydride, I is converted to 4,4,6-trimethyl-4H-1,3-thiazine-2-thiol (II). It has been further demonstrated that the same sequence of reactions, with the formation of the same compounds, takes place in method B where 2-methyl-2-isothiocyano-4-pentanone and dithiocarbamic acid react together. Although only hydrogen sulfide (contributed by dithiocarbamic acid) is required for this reaction, attempts to prepare I by direct reaction of hydrogen sulfide and 2-methyl-2-isothiocyano-4-pentanone were unsuccessful.



The ring structure Ib is preferred for the intermediate since infrared absorption showed only a weak band at 5.5 to 6 μ which could be attributed to the carbonyl group. In addition, attempts to form a dinitrophenylhydrazone were unsuccessful. A comparison of ultraviolet absorption for I with that for the corresponding dihydrothiazine, 4,4,6-trimethyl-4,5-dihydro-4H-1,3-thiazine-2-thiol (III),[1] shows that they are in fairly close agreement (Table I). This further supports the ring structure Ib.

Experimental[3]

TABLE I

Compound	Spectrum[a] Max.	Spectrum[a] Min.
I (meth. A)	^{a}285.5 = 60.5 ^{a}240.5 = 29.5	^{a}257.8 = 10.6
I (meth. B)	^{a}285.5 = 65.1 ^{a}240.0 = 33.6	^{a}257.7 = 12.9
II	^{a}296.0 = 69.4	
III[b]	^{a}286.5 = 77.6 ^{a}241 = 42.6	^{a}258 = 10.2

[a] Determinations were made in methanol solution using a Beckman model DK-2 spectrophotometer. [b] Values are at a small variance with those previously reported (reference 1) where a Beckman model DU spectrophotometer was used.

2-Methyl-4-oxopentyldithiocarbamic Acid (I). Method A.—Diacetoneamine[4] (46 g., 0.4 mole), as a 60% aqueous solution, and 31 g. of carbon bisulfide (0.4 mole) were vigorously stirred at room temperature. After about an hour, a yellow, crystalline compound precipitated; agitation was continued for another hour. The solid was filtered, washed and dried to give 63 g. (83%). Purification was effected by recrystallization from acetone. An analytical sample melted at 114° dec.[5] After storage for about three weeks, I decomposed by partially liquefying and evolving hydrogen sulfide and carbon bisulfide.

Anal. Calcd. for $C_7H_{13}NOS_2$: C, 43.93; H, 6.85; N, 7.33. Found: C, 44.08; H, 6.87; N, 7.33.

In a less satisfactory alternative method, the experiment described was repeated using the same weight of reactants, and in addition 17 g. (0.4 mole) of sodium hydroxide. The red-colored solution obtained, which consisted of a solution of the sodium salt of I, was acidified. There was obtained 37 g. (49%) of I as determined by the unchanged melting point of a mixture.

4,4,6-Trimethyl-4H-1,3-thiazine-2-thiol (II).—To 147 g. (0.75 mole) of freshly prepared I, there was added 225 g. of 70% sulfuric acid. This mixture was heated to 85°, and the slurry was added to one liter of ice-water. The solid was filtered, washed free from acid and dried to give 129 g. (96%) of crude product melting at 114–118°. After recrystallization from carbon tetrachloride, an analytical sample melted at 120.5–121.5°.

Anal. Calcd. for $C_7H_{11}NS_2$: C, 48.51; H, 6.40; N, 8.08; S, 37.00; mol. wt., 173. Found: C, 48.81; H, 6.38; N, 7.99; S, 36.57; mol. wt., 177.

An identical compound was obtained by heating I with acetic anhydride to which a drop of sulfuric acid had been added.

2-Methyl-4-oxopentyldithiocarbamic Acid (I). Method B.—A mixture of 31.4 g. (0.2 mole) of 2-methyl-2-isothiocyano-4-pentanone,[6] 18 cc. (0.22 mole) of hydrochloric acid and 50 cc. of 50% ethanol was stirred vigorously while adding 26.5 g. (0.24 mole) of ammonium dithiocarbamate as a 45% aqueous solution. The addition time was about 20 minutes, and cooling was necessary to maintain the reaction mixture at room temperature. The product was present as an insoluble, red liquid and continued stirring for 3 hours converted the liquid to soft pellets. The solid was filtered, washed with water and further washed with petroleum ether which was effective in removing the oiliness. The crude product weighed 27 g. (71%), and melted at 114–114.5° after purification by recrystallization from acetone. This compound was shown to be the same as I prepared by method A by means of ultraviolet absorption spectra which were in good agreement.

Anal. Found: C, 44.23; H, 7.00; N, 7.57.

Since only hydrogen sulfide (contributed by dithiocar-

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(1) J. E. Jansen and R. A. Mathes, THIS JOURNAL, **77**, 2866 (1955).

(2) S. Gabriel and T. Posner, *Ber.*, **27**, 1037 (1894).

(3) All melting points are uncorrected.

(4) H. Gilman, Editor, "Organic Syntheses," Coll. Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1941, p. 196. For the purpose of this work, it was not necessary to prepare the oxalate as described. A water solution of diacetoneamine can be temporarily stored in a refrigerator with little change.

(5) Gabriel and Posner found m.p. 119–120°.

(6) R. A. Mathes, THIS JOURNAL, **75**, 1747 (1953).

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.

Acknowledgment.—The analyses of compounds were carried out by J. R. Kubik and A. K. Kuder. Infrared and ultraviolet absorption work was performed by J. J. Shipman and J. P. Kiehl.

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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

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

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 constants $K_\mathrm{S} = (k_2 + k_3)/k_1$ 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.

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

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

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

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

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

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.

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

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

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

$(\mathrm{[S]_0 - [S]_t})/t$ *vs.* $(\ln (\mathrm{[S]_0/[S]_t}))/t$ 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 $(\mathrm{[S]_0 - [S]_t})/t$ 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.

[2] H. Lineweaver and D. Burk, THIS JOURNAL, **56**, 658 (1934).

[3] G. S. Eadie, *J. Biol. Chem.*, **146**, 85 (1942).

[4] A. C. Walker and C. L. A. Schmidt, *Arch. Biochem.*, **5**, 445 (1943).

[5] R. R. Jennings and C. Niemann, THIS JOURNAL, **75**, 4687 (1953).

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

[7] T. H. Applewhite and C. Niemann, THIS JOURNAL, **77**, 4923 (1955).

[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.