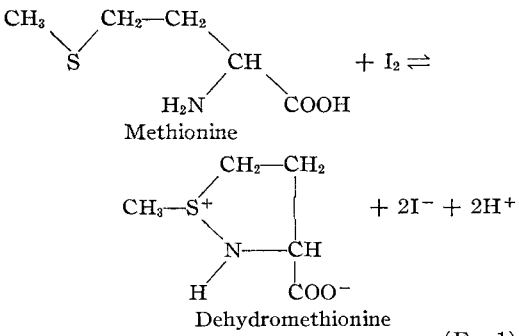
Kinetic Investigation of Reversible Reaction Between Methionine and Iodine

Improved Iodometric Determination of Methionine

By KARL-HEINZ GENSCH* and TAKERU HIGUCHI

Rate studies of reactions discovered by Lavine involving reversible oxidation of methionine to dehydromethionine with iodine are presented. The data suggest that the initial iodine attack may occur either on the nitrogen or on the sulfur. The hydrolytic rate of dehydromethionine to the corresponding sulfoxide appears to be sufficiently fast to cause some inherent errors in the original method of assay for the amino acid.

ALTHOUGH methionine has been determined by a number of iodometric methods (1–5), the chemistry involved has not been clearly established. A method of assay was proposed by Lavine (1) in 1943 based on the following reaction:



(Eq. 1)

Methionine reacts reversibly with iodine to form a cyclic sulfonium compound which Lavine called dehydromethionine (1). According to the method, methionine is allowed to react in a buffered neutral solution with an excess of iodine for 20 min. After removal of the unreacted iodine by careful addition of sodium thiosulfate solution, sufficient potassium iodide and hydrochloric acid (to pH 0.5 to 1.0) are added to reverse the reaction. The liberated iodine is finally titrated with a standardized sodium thiosulfate solution from which the amount of methionine can be calculated. This method suffers from two important disadvantages: (*a*) it is time consuming because of several operations, and (*b*) it cannot theoretically yield stoichiometrically correct results as will be shown later. Several modifications of the original Lavine method have been proposed, but they also require relatively long times for analysis (2–5).

The present study was undertaken to elucidate the microscopic chemistry of this interesting reaction and to provide possible leads for improvement in the method. As parts of these investigations the dependences of the reaction rates in both directions on pH, iodide-ion concentration, and iodine concentration have been determined. The hydrolysis of the cyclic oxidation product has also been studied. A possible improved method of analysis of the acid is proposed as a result of this investigation.

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RESULTS AND DISCUSSION

Rate of Oxidation of Methionine.—*Forward Reaction.*—The rate of oxidation of methionine with iodine to form dehydromethionine can be conveniently followed spectrophotometrically at 353 mμ, the absorbance peak of I_3^-. From pseudo first-order rate constants obtained in presence of excess methionine and essentially constant iodide and hydrogen-ion concentrations, observed second-order rate constants for the forward reaction, $k_{f(obs.)}$ can be readily calculated by dividing by the concentration of the amino acid. Or

$$-\frac{d[I_2]_T}{dt} = k_{f(obs.)}\,[Me]_T[I_2]_T \quad \text{(Eq. 2)}$$

where Me represents methionine, and the subscript, T, designates the total concentration of both the titratable iodine and methionine.

In Fig. 1 the observed second-order rate constants obtained at pH 7 in this manner have been plotted against the reciprocal of the iodide concentration. It is evident from the observed linear relationship that the forward rate is inversely proportional to the iodide concentration in the system. This behavior can be best rationalized on the basis that free iodine is involved in this mechanism since, according to the equilibrium in Eq. 3, the concen-

$$I_2 + I^- \overset{K_I}{\rightleftharpoons} I_3^- \quad \text{(Eq. 3)}$$

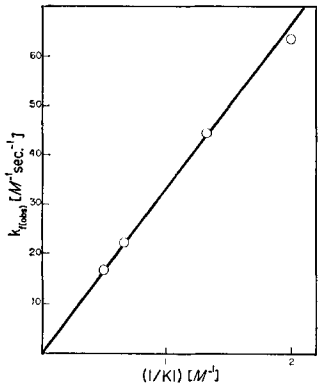


Fig. 1.—Observed second-order rate constants *vs.* (1/KI) for the oxidation of methionine with iodine at pH 7.2. Initial $C_5H_{11}NO_2S$ = 6.82 × 10^{-4} *M*; initial total I_2 = 10^{-4} *M*; phosphate buffer = 0.02 *M*; ionic strength = 2.05 (adjusted with KCl); temperature = 25.0°.

tration of free iodine is inversely proportional to that of iodide at higher iodide concentrations.

In Fig. 2 the product of observed second-order rate constants obtained in the above fashion and the iodide concentration for each run have been plotted as a function of the pH of the system. Different iodide concentrations were used to adjust the observed rate to a convenient range. From the profile, it is evident that the rate is essentially inversely proportional to the hydrogen concentration in the range between pH 4 and 8. Above pH 8 the curve levels off, the observed second-order rate constant at pH 10 being approximately twice that at pH 9. Since the pKa_2 of methionine in this system is approximately 9, the observed pH dependence strongly suggests that the free amino group is involved in this reaction.

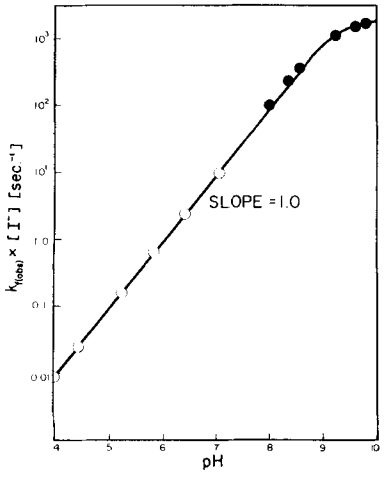


Fig. 2.—Products of observed second-order rate constant and iodide concentration *vs.* pH for the oxidation of methionine with iodine. Initial $C_5H_{11}NO_2S$ = 5 × 10^{-4} *M*; initial total I_2 = 10^{-4} *M*; phosphate or phthalate buffer = 0.02 *M*; ○ = $k_{f(obs.)}$ measured in 0.1 *M* KI, ● = $k_{f(obs.)}$ measured in 1 *M* KI; temperature = 25.0°.

On the basis of the results presented in Figs. 1 and 2 the following rate law can be written:

$$\frac{-d[I_2]_T}{dt} = k_{f(obs.)}\,[Me]_T\,[I_2]_T$$

$$= k_f[Me^-]\,[I_2] \quad \text{(Eq. 4)}$$

where Me^- represents the anionic methionine species and k_f is the second-order rate constant for the species Me^- and I_2 of the forward reaction. Considering Eq. 2 and the dissociation of methionine as expressed by

$$Ka_2 = \frac{[Me^-]\,[H^+]}{[Me]} \quad \text{(Eq. 5)}$$

we can write the forward rate as

$$\frac{-d[I_2]_T}{dt} = \frac{k_f Ka_2\,[Me]_T[I_2]_T}{([H^+] + Ka_2)\,(1 + K_I[I^-])} \quad \text{(Eq. 6)}$$

and for the observed second-order rate constant as follows

$$k_{f(obs.)} = \frac{k_f Ka_2}{([H^+] + Ka_2)\,(1 + K_I[I^-])} \quad \text{(Eq. 7)}$$

From Eq. 7 and the data as shown in Figs. 1 and 2, a mean value for k_f at 25° was determined to be 6.5 × 10^5 M^{-1} sec.$^{-1}$.

Rate of Reduction of Dehydromethionine.—*Reverse Reaction.*—The reduction of the cyclic species by iodide was readily followed spectrophotometrically by the appearance of the I_3^- species. Pseudo first-order rate constants, for the reverse reaction, $k_{r(obs.)}$, were obtained from such data in either of two ways. For fast reaction at constant pH and excess iodide concentration, pseudo first-order rate constants were obtained by plotting log $(A_\infty - A)$ against time. If the rate was expected to be slow, a relatively high concentration of dehydromethionine was used, and the initial rate of formation was measured. Based on previously determined values for the molar absorptivity of iodine in the particular system, the initial rate of reaction was converted to pseudo first-order rate constants by dividing through by the initial dehydromethionine concentration.

Pseudo first-order rate constants obtained in this fashion at different pH values are shown plotted *versus* pH in Fig. 3. From the resulting pH profile it is evident that above pH 4.5 the rate is directly proportional to the hydrogen-ion concentration, while in the more acidic region the order of the over-all reaction with respect to the hydrogen concentration is larger than 1.

The dependence of the reverse pseudo first-order rate constant on the iodide concentration at pH of 5.02 is evident from the log-log data plotted in Fig. 4. The line which closely fits the experimental points was drawn with a slope of 2. On this basis, it would appear that the kinetic dependence with respect to iodide is 2. Similar determinations at pH 2.48, however, yielded a log-log plot with a slope of 1.52 also shown in Fig. 4. From these studies it would appear that the responsible reaction pathway in the acidic region may differ at least in part from that operating at neutral pH. If we assume that the protonated dehydromethionine requires an attack by only a single iodide anion in the rate-determining step while the

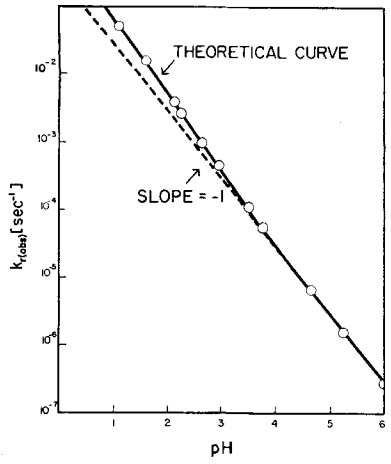


Fig. 3.—Observed first-order rate constant *vs.* pH for the reduction of dehydromethionine. KI = 0.1 *M*; $C_5H_9NO_2S$ = 8.3 × 10^{-5} to 10^{-2} *M*; phosphate or acetate buffer = 0.016 *M*; the theoretical curve was calculated from Eq. 10. The constants were determined by trial and error to be k'_r = 27 [M^{-3} sec.$^{-1}$], k''_r = 3 × 10^{-3} [M^{-2} sec.$^{-1}$], Ka = 3 × 10^{-3} [*M*] at 25.0°.

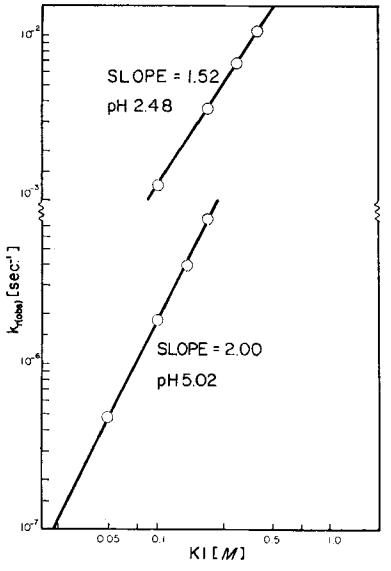


Fig. 4.—Log-log plot of observed first-order rate constant *vs.* potassium iodide for the reduction of dehydromethionine. At pH 5.02: succinate buffer = 0.1 *M*; $C_5H_9NO_2S$ = 0.01 *M*; ionic strength = 0.35 (adjusted with KCl). At pH 2.48: phosphate buffer = 0.01 *M*; $C_5H_9NO_2S$ = 5 × 10^{-5} *M*; ionic strength = 0.41 (adjusted with KCl); temperature = 25.0°.

neutral species requires two, the following rate law can be written:

$$\frac{d[I_2]_T}{dt} = k_{r(obs.)}[De]_T = k_r'[De][H^+][I^-]^2 + k_r''[DeH^+][H^+][I^-] \quad \text{(Eq. 8)}$$

where $[De]_T$, [De], and $[DeH^+]$ represent the concentration of the total, unprotonated, and protonated dehydromethionine, respectively. The constants k_r' and k_r'' are fourth and third-order rate constants, respectively, for these different mechanisms. Since the dissociation constant of the protonated dehydromethionine is

$$Ka = \frac{[De][H^+]}{[DeH^+]} \quad \text{(Eq. 9)}$$

from Eq. 8 the following rate law can be derived:

$$k_{r(obs.)} = \frac{k_r'Ka[H^+][I^-]^2}{[H^+] + Ka} + \frac{k_r''[H^+]^2[I^-]}{[H^+] + Ka} \quad \text{(Eq. 10)}$$

The constant k'_r can be easily determined from the pH profile in Fig. 3, the apparent pseudo first-order rate constant being, above pH 4.5,

$$k_{r(obs.)} \cong k_r'[H^+][I^-]^2 \quad \text{(Eq. 11)}$$

A mean value of k_r', the rate constant for the reverse reaction in essentially neutral system, was calculated on this basis to be 27 [M^{-3} sec.$^{-1}$] at 25°.

From the shape of the pH profile and from Eq. 10, we can estimate the dissociation constant of the protonated dehydromethionine and the rate constant for the reverse reaction in acid solution to be Ka = 3 × 10^{-3} [M^1] and k_r'' = 7 [M^{-2} sec.$^{-1}$].

Although the dissociation constant was not measured directly, the obtained pKa value of 2.52 appears reasonable on another basis. From the structure point of view, a pKa value not too different from that of methionine (pKa = 2.22) appears to be likely because the positive sulfur in dehydromethionine may be expected to increase the dissociation of the carboxyl group as the protonated amino group in methionine does. Furthermore, the pH profile of the hydrolysis of dehydromethionine to methionine sulfoxide (Fig. 10), as will be discussed later, corresponds to the same value based on the assumption that the protonated dehydromethionine hydrolyzes with a different rate than the neutral species.

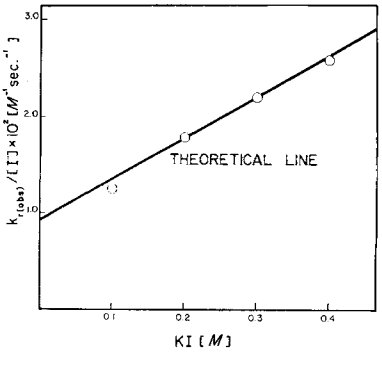


Fig. 5.—According to Eq. 12, observed first-order rate constants obtained at pH 2.48 (see Fig. 4) for the reduction of dehydromethionine divided by the iodide concentration are shown plotted against the iodide concentration. The theoretical curve was calculated from Eq. 12 using the constants showing best fit for pH profile in Fig. 3.

The constants k_r', k_r'', and Ka can also be shown to be internally consistent with the observed rate dependence on the iodide concentration. From Eq. 10 the linear relationship

$$\frac{k_{r(obs.)}}{[I^-]} = \frac{k_r'Ka[H^+][I^-]}{[H^+] + Ka} + \frac{k_r''[H^+]^2}{[H^+] + Ka} \quad \text{(Eq. 12)}$$

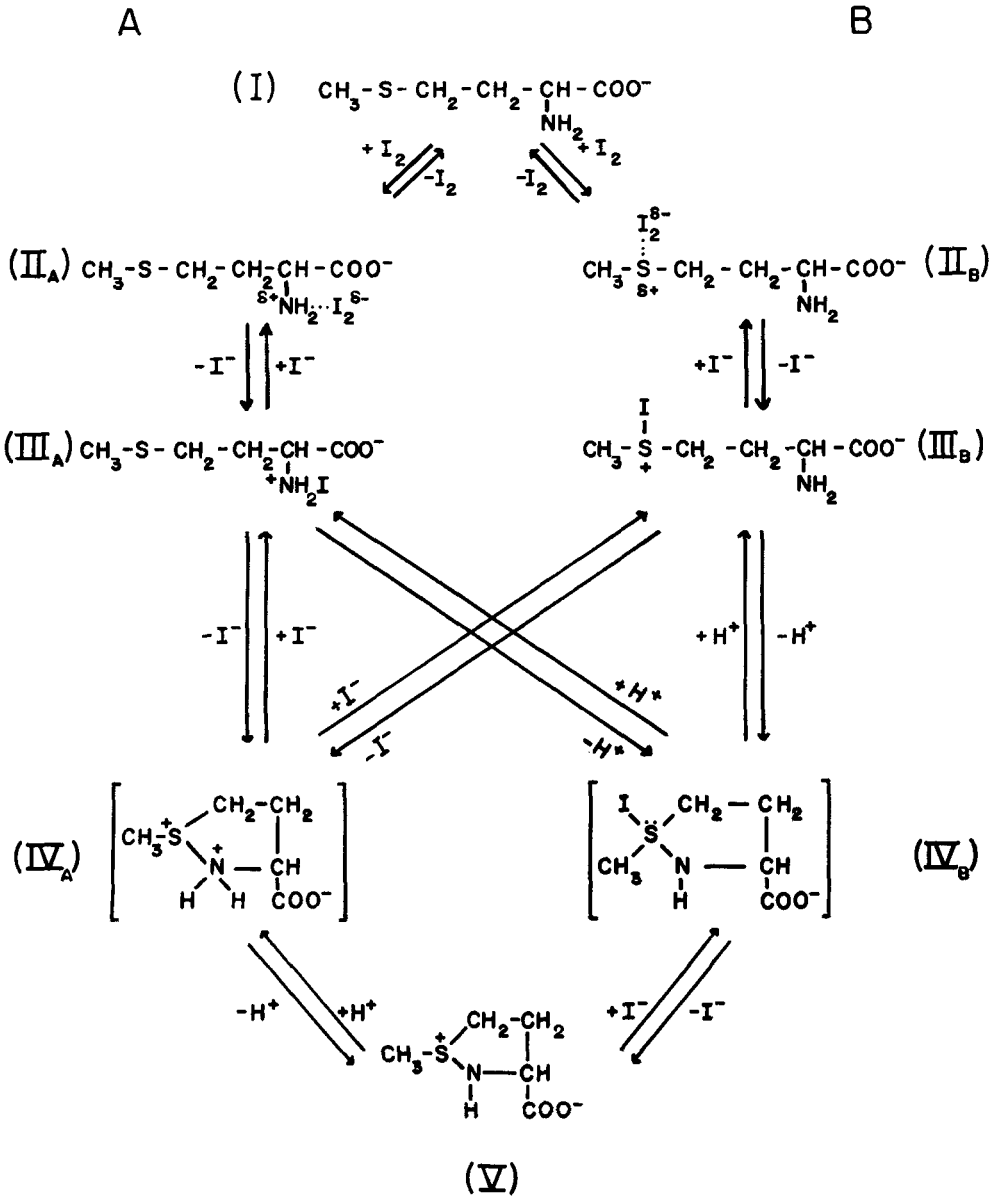
can be derived.

By plotting $k_{r(obs.)}/[I^-]$ against the iodide concentration of a particular system for the reduction of dehydromethionine, the data can be expected to fall on a straight line with a slope of $k_r''Ka[H^+]/([H^+] + Ka)$ and an intercept of $k_r''[H^+]^2/([H^+] + Ka)$. Such a plot is presented in Fig. 5 for data obtained at various iodide concentrations at pH 2.48. The reasonable fit of the theoretical line calculated from Eq. 12 and the constants obtained from Fig. 3 suggest that the proposed mechanism can account for the observed fractional iodide dependence at lower pH.

GENERAL DISCUSSION

Since free iodine has been shown and would be expected to interact with both amines (6) and alkyl sulfides (7), both sites of initial interaction must be considered. Possible reaction schemes based on these routes are diagrammed in Scheme I.

Pathway A considers an attack of iodine on the amino group by which the iodine amine complex



Scheme I

II_A might be formed. Dissociation to III_A seems to be very likely; the further pathway, however, might go either through the species IV_A or IV_B, both of which must be regarded as suitable. Considering the iodination of the amino group to be the rate-determining step, the rate law for the forward reaction would agree with this assumption. Also, the rate law for the reverse reaction is in agreement with pathway A; however, the formation of IV_A in neutral solution and further attack by iodide to form III_A appears to be not very likely. Also, the alternative in the mechanism going from IV_B to III_A cannot be easily accepted because it would require a fast migration of iodine from sulfur to nitrogen for which no support can be presented.

If an iodine attack on sulfur is assumed, as shown in pathway B, the reverse reaction could be more easily explained; the forward reaction, however, would only fit the rate law if additional assumptions were made. Studies with simple aliphatic thioether have revealed (8) that the rate of oxidation by iodine forming sulfoxide is inversely proportional to the square of the iodide concentration. For this reaction, the mechanism in Scheme II is proposed.

$$I_2 + I^- \rightleftharpoons I_3^-$$

$$R{-}S{-}R' + I_2 \rightleftharpoons R{-}\overset{+}{S}(I){-}R' + I^-$$

$$R{-}\overset{+}{S}(I){-}R + OH^- \rightarrow R{-}S(=O){-}R' + H^+ + I^-$$

Scheme II

If in the forward reaction also an iodosulfonium-cation of methionine is formed, the over-all rate, too, should be inversely proportional to the square of the iodide concentration. However, this has not been observed. This contradiction might be removed if one assumes that the dissociation from II_B to III_B is relatively slow or occurs with about the same rate as the cyclization of III_B. The reverse reaction may go through a nucleophilic attack of dehydromethionine by iodide, forming IV_B which on protonation goes under ring opening to the iodosulfonium species III_B. By a second attack of iodide, as the rate law requires, methionine and iodine are formed, the latter is greatly complexed with I^- to form I_3^-. Although pathway V $\rightarrow$ $IV_A \rightarrow III_B$ is also possible, it seems unlikely in neutral solution.

Some support for a mechanism based on attack of iodine on sulfur is evident in the behavior of the forward reaction in the presence of very low iodine, very low methionine, and very low iodide concentration and at pH values not higher than 5.50. The observed second-order rate constants obtained in these studies multiplied by $(1 + K_I [I^-])(1 + [H^+]/Ka_2)$ are shown in Fig. 6 against the reciprocal of the iodide concentration ranging from 3.3×10^{-4} to 1.66×10^{-3} *M* K_I. If Eq. 6 would be the true rate law under these conditions, a rate corrected rate constant should be independent of iodide. The graph, however, shows straight lines with intercepts approximately equal to k_f and with slopes which increased with increasing hydrogen-ion concentration. Although this behavior is not very well understood, it appears likely that at lower pH, where the concentration of Me^- is very low, positive iodosulfonium species is involved in this reaction.

Hydrolysis of Dehydromethionine.—Besides the reduction reaction discussed above, in the presence of iodide and hydrogen ions, dehydromethionine appears to undergo hydrolysis to methionine sulfoxide which is more difficult to reduce than dehydromethionine itself. A critical investigation of the Lavine method must, therefore, also include a kinetic study of this competitive hydrolysis in the absence and in the presence of buffer species.

The rates of hydrolysis in the absence and in the presence of different buffer species have been determined by withdrawing periodic samples from a stock solution of dehydromethionine and titrating the liberated iodine with sodium thiosulfate solution after addition of potassium iodide and hydrochloric acid. Plots of log (ml. 0.01 *N* $Na_2S_2O_3$) *versus* time yielded

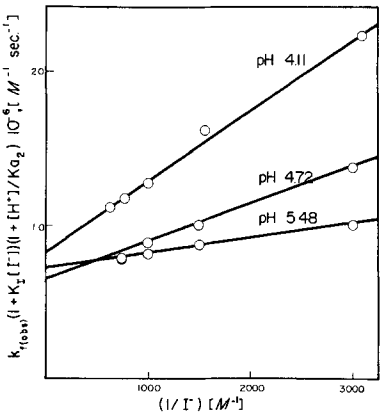


Fig. 6.—Plot showing the change of the dependence of the forward rate on the iodide concentration at different pH values. Initial total iodine = 5×10^{-6} *M*; initial total methionine = 4×10^{-4} *M*; succinate buffer = 0.002 *M*; temperature = 25.0°.

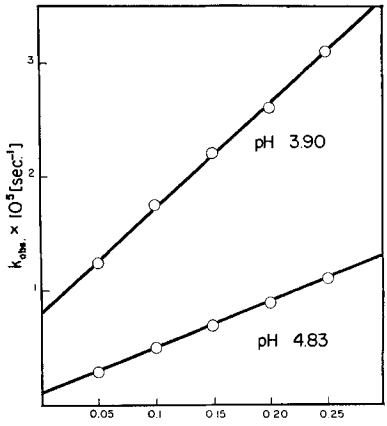


Fig. 7.—Observed first-order rate constants *vs.* total acetate buffer concentration for the hydrolysis of dehydromethionine (measured titrimetrically). Buffer ratio = 0.3 mole CH_3COO^-/0.7 mole CH_3COOH at pH 3.90; 0.7 mole CH_3COO^-/0.3 mole CH_3COOH at pH 4.83. $C_5H_9NO_2S$ = 0.01 *M*; ionic strength = 0.875 (adjusted with KCl); temperature = 25.0°.

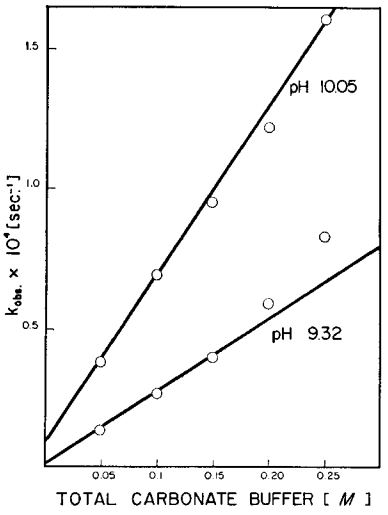


Fig. 8.—Observed first-order rate constants *vs.* total carbonate buffer concentration for the hydrolysis of dehydromethionine (measured titrimetrically). Buffer ratio = 0.3 mole HCO_3^-/0.7 mole $CO_3^=$ at pH 10.05; 0.7 mole HCO_3^-/0.3 mole $CO_3^=$ at pH 9.32. $C_5H_9NO_2S$ = 0.01 *M;* ionic strength = 0.875 (adjusted with KCl); temperature = 2.50°.

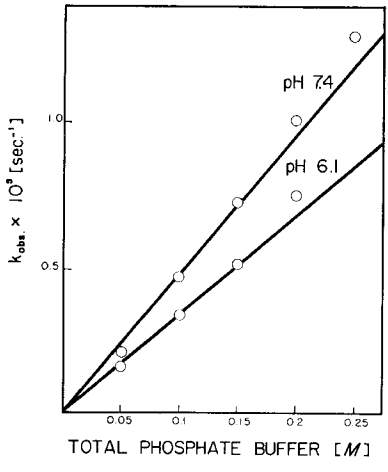


Fig. 9.—Observed first-order rate constants *vs.* total phosphate buffer concentration for the hydrolysis of dehydromethionine (measured titrimetrically). Buffer ratio: 0.15 mole $H_2PO_4^-$/0.85 mole $HPO_4^=$ at pH 7.4; 0.7 mole $H_2PO_4^-$/0.3 mole $HPO_4^=$ at pH 6.1. $C_5H_9NO_2S$ = 0.01 *M;* ionic strength = 0.875 (adjusted with KCl); temperature = 25.0°.

straight lines from which observed pseudo first-order rate constants could be calculated. In strongly acidic solutions, the rate was also measured spectrophotometrically by following the decrease in absorbance at 243 mμ.

Figures 7, 8, and 9 show the influence of the different buffer species on the rate of hydrolysis of dehydromethionine. In these graphs the observed pseudo first-order rate constants obtained in presence of acetate, carbonate, and phosphate buffer are shown plotted against the total buffer concentration. For most plots the experimental points fall essentially on a straight line, the intercepts representing pseudo first-order rate constants at the particular pH in absence of any buffer and the slopes the specific second-order rate constants for the buffer species involved.

$$k_{obs.} = k_{H_2O} + k_T\,[\text{buffer}]_T \quad \text{(Eq. 13)}$$

Since each buffer mixture consisted of an acid and a basic species, the following equation for the slope can be written

$$k_T = k_{base} \times F_{base} + k_{acid} \times F_{acid} \quad \text{(Eq. 14)}$$

where F represents the fraction of the basic or acidic species, *i.e.*, $F_{base} + F_{acid} = 1$. Since for each buffer system two series of experiments with different buffer ratios were carried out from the slopes of these two plots and the particular fractions, the catalytic second-order rate constant for the acidic species and the basic species was calculated. These rate constants are summarized in Table I.

The rate of hydrolysis in absence of any buffer has also been studied, mainly at low and at high pH, the hydrogen-ion concentration was kept constant by repeatedly adding sodium hydroxide solution. The results of these studies and the extrapolated values for zero buffer concentration of Figs. 7, 8, and 9 are presented as the pH profile in Fig. 10 showing that dehydromethionine hydrolyzes easily in presence of hydrogen ions and hydroxide ions and is very stable at neutral pH ($t_{1/2} \approx 600$ days).

As can be seen in Fig. 10, the rate of hydrolysis in strong acid solution is about only a third of the rate which could be expected by extrapolation from the observed values at pH 5. This decrease of the observed rate constants may be due to the slower rate of reaction of the protonated species relative to the free form. On the basis of this assumption, the following rate law for the hydrolysis of dehydromethionine in acidic solution and in the absence of any buffer species can be written:

$$\frac{-d[\text{De}]_T}{dt} = k_{obs.}\,[\text{De}]_T =$$

$$k_{H^+}[\text{De}]\,[H^+] + k'_{H^+}\,[\text{DeH}^+]\,[H^+] \quad \text{(Eq. 15)}$$

Considering Eq. 9, the observed first-order rate constant can be expressed as

$$k_{obs.} = \frac{k_{H^+}\,\text{Ka}\,[H^+] + k'_{H^+}\,[H^+]^2}{[H^+] + \text{Ka}} \quad \text{(Eq. 16)}$$

From Eq. 16 and the experimental data of Fig. 10, the constants were determined to be $k_{H^+} = 6 \times 10^{-2}$ [M^{-1} sec.$^{-1}$], $k'_{H^+} = 1.7 \times 10^{-2}$ [M^{-1} sec.$^{-1}$], and Ka = 3×10^{-3} [*M*] which agrees with the value obtained from Fig. 3. The theoretical curve of

TABLE I.—CATALYTIC CONSTANT FOR HYDROLYSIS OF DEHYDROMETHIONINE FOR SEVERAL BUFFER SPECIES FROM FIGS. 7, 8, AND 9 AT 25°

Catalytic Active Species	k [M^{-1}sec.$^{-1}$] × 10^4
CH_3COOH	1.31
CH_3COO^-	0.0013
$H_2PO_4^-$	0.25
$HPO_4^=$	0.5
HCO_3^-	0.09
$CO_3^=$	8.6

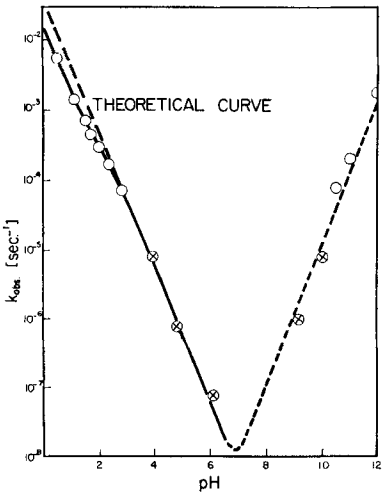


Fig. 10.—Observed first-order rate constants *vs.* pH for the hydrolysis of dehydromethionine (measured titrimetrically and spectrophotometrically). Key: ○, determined directly; ⊗, determined by extrapolation from Figs. 7, 8, and 9. $C_5H_9NO_2S$ = 0.01 *M;* ionic strength = 0.875 (adjusted with KCl); temperature = 25.0°. The theoretical curve was calculated from Eq. 16, derived from the assumption of different rates for unprotonated and protonated dehydromethionine, values of k_{H^+}, k'_{H^+}, and Ka of Eq. 16 were chosen to fit best the experimental points.

Fig. 10 is calculated from Eq. 16 and is in good agreement with the experimental results.

The mechanism of the hydrolysis of dehydromethionine in alkali solution seems to be best rationalized by assuming a nucleophilic attack of hydroxide ion or anionic buffer species on the positive sulfur, followed by ring opening and protonation of the nitrogen. In acidic solution protonation of the imino group probably occurs first, followed by a water attack on sulfur. The relatively high catalytic activity of acetic acid compared to the acetate species might be caused through a nucleophilic attack of the carbonyl oxygen on the sulfur. Protonation of the imino group occurs then through general acid catalysis of the attached carboxyl group.

The influence of iodide ions on the rate of hydrolysis of dehydromethionine as mentioned by Lavine (1) was not observed. It seems to be likely that Lavine was not aware of the catalytic activity of phosphate buffer.

Application of Kinetics Leading to Improvement of Assay Method.—In the original method proposed by Lavine, dehydromethionine stands for 20 min. in contact with 0.2 *M* phosphate buffer at pH 7. It is evident from the data given above on the hydrolysis of dehydromethionine that a significant portion will be observed to form a sulfoxide species which would not undergo reversible reduction back to the amino acid. The extent of loss on the basis of our experiment would be of the order of 1%. Another small error of about 0.2% in the presence of 1 *M* potassium iodide solution and at pH 1 can be calculated from Eqs. 10 and 16 because the reduction and the hydrolysis of dehydromethionine are competitive reactions.

From the relative high value of the specific second-order rate constant $k_f = 6.5 \times 10^5$ $[M^{-1}$ sec.$^{-1}]$ of the forward reaction, it could be expected that at about pH 9 and in presence of low potassium iodide concentration methionine can be directly titrated with iodine. This possibility has already been mentioned by Lavine (1); no result, however, has been presented. The following procedure was tested with the same lot of methionine.

About 150 mg. methionine, previously dried at 60° in vacuum for 2 hr., was accurately weighed and dissolved in 50 ml. of water, with 1 Gm. of sodium borate. After addition of 1 ml. of 1% starch solution, it was titrated with 0.1 *N* iodine until a slightly blue color remained for at least 1 min.

The results are given in Table II for 9 separate analyses and compared with 9 independent determinations of the same sample according to the procedure of N.F. XII.

Both methods have practically the same precision judged by the standard deviation. The differences of about 0.5% between the mean values seems to be partially due to the different ways by which the end point is approached. In the direct iodometric titration a slight excess of iodine is added to produce the starch–iodine complex, while for the back titration of iodine with sodium thiosulfate, a slight excess of the titrant is unavoidable. Therefore, the difference of about 0.5% includes the sum of the titration errors of the two methods. Both methods were also carried out in the presence of glycine (equivalent to 10% of the applied amount of methionine). No higher values were observed.

The value obtained by direct titration can also be checked by continuing with an indirect determination of methionine in the same solution.

The slight excess of iodine is carefully removed by sodium thiosulfate solution, 12 Gm. of potassium iodide and 10 ml. of 25% hydrochloric acid are added, and the liberated iodine is titrated with 0.1 *N* sodium thiosulfate solution. Since in a solution of 1 *M* potassium iodide at low pH the iodide species can easily be oxidized by air, a blank is performed by preparing a solution of 1 Gm. sodium borate, 11 Gm. potassium iodide, and 10 ml. of 25% hydrochloric acid in 70 ml. of water and titrating the liberated iodine after 1 to 2 min. of stirring.

The mean value of the indirect determination and its standard deviation is also given in Table II and shows very good agreement with the direct titration. Since this indirect procedure, however, shows no advantages, the direct iodometric method appears to be sufficient and also superior to the N.F. XII assay, because it can be carried out in much shorter time.

TABLE II.—RESULTS OF A NEW DIRECT AND INDIRECT IODOMETRIC METHOD OF ASSAY OF METHIONINE COMPARED WITH THE DETERMINATION IN "THE NATIONAL FORMULARY," 12TH ED.

Procedure	No. of Determinations	Mean Value, %	S. D., %
Direct	9	100.17	±0.22
Indirect	9	100.00	±0.25
N.F. XII	9	99.68	±0.24

EXPERIMENTAL

Reagents.—Methionine (Eastman-Kodak, white label) was recrystallized from 40% ethanol. All other reagents were analytical grade. Water was purified by distilling tap distilled water from a potassium permanganate solution acidified with sulfuric acid.

The rate of oxidation of methionine with iodine and the rate of reduction of dehydromethionine by iodide forming iodine have been determined spectrophotometrically with a Cary model 11 or 15 by measuring the change in absorbance at 353 mμ, the absorption peak of I_3^-.

For the forward reaction, in general 5 ml. of a methionine solution was added to 45 ml. of a solution of buffer, potassium iodide, and iodine to yield 50 ml. of a solution with concentration as given for each figure. Very fast reactions were measured by injecting 1 ml. of methionine solution through the cover of the cell compartment into 2 ml. of solution containing buffer, iodide, and iodine while the instrument was operating.

For studying the reverse reaction, a stock solution of 0.025 *M* dehydromethionine was prepared by adding an equivalent amount of iodine and sodium hydroxide solution to a methionine solution, the pH was kept between pH 5 and 7. Iodide was removed with silver nitrate, the filtrate was brought to the calculated volume. Pseudo first-order rate constants were obtained as described above in the section for the reverse reaction.

The rate of hydrolysis of dehydromethionine was determined in 0.01 *M* solution of the cyclic species in the presence and in the absence of the buffer species. In both cases 10-ml. samples were withdrawn from the thermostated stock solution, 3 Gm. potassium iodide and 10 ml. 0.5 *N* hydrochloric acid were added, and the liberated iodine was titrated with 0.01 *N* sodium thiosulfate solution. From a plot of log (ml. 0.01 *N* $Na_2S_2O_3$) *versus* time, pseudo first-order rate constants were obtained.

Since dehydromethionine hydrolyzes under acid formation, the rate of hydrolysis in the absence of any buffer was carried out under simultaneous addition of sodium hydroxide solution. For this purpose the reaction solution was kept in a water jacket container closed with a ground-glass plate. Through the holes in the glass plate, electrodes and the tip of a microburet filled with 1 *N* sodium hydroxide solution were immersed into the solution stirred by a magnetic stirrer. Since for most cases a half-life of more than 10 min. was expected, the pH could be easily controlled manually.

In strong acidic solution the rate of hydrolysis was also determined spectrophotometrically by following a decrease in absorbance at 243 mμ.

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Coumarins IV

Coumarins of *Pteryxia terebinthina*. Structures of Two New Coumarins, Isopteryxin and Calipteryxin

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The ether extract of the root of *Pteryxia terebinthina* var. *californica* (Coult. and Rose) Mathias, in addition to osthol, anomalin (I), and pteryxin (III), afforded two new coumarins: 3′(*S*),4′(*S*)-3′-angeloyloxy-4′-acetoxy-3′,4′-dihydroseselin (IV) and 3′(*S*),4′(*S*)-3′-angeloyloxy-4′-senecioyloxy-3′,4′-dihydroseselin (V). These coumarins are provisionally named isopteryxin and calipteryxin, respectively.

In keeping with an interest in naturally occurring coumarins (1) and prompted by the recent isolation of anomalin (I) from *Angelica anomala* Lall by Hata *et al.*, and its structure determination as the 3′,4′-diangelate ester of 3′,4′-dihydroxy-3′,4′-dihydroseselin (II), the present paper reports the isolation of I and several related coumarins from *Pteryxia terebinthina* var. *californica* (Coult. and Rose) Mathias.[1] In particu-

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