May, 1982] © *1982 The Chemical Society of Japan* *Bull. Chem. Soc. Jpn.*, ***55***, *1493—1497 (1982)* 1493

Reactions of Thiols with Phenylglyoxal to Give Thiomandelic *S*-Esters. Formation of Hemithioacetals and Their Rearrangement

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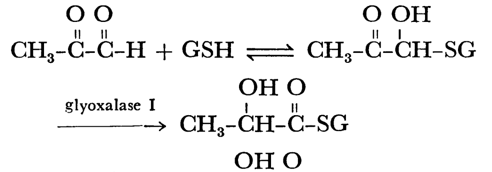
(Received April 30, 1981)

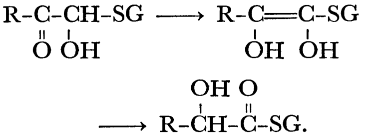
Equilibrium constants K_h for the addition of 2-mercaptoethanol and glutathione to phenylglyoxal to form hemithioacetals were determined spectrophotometrically over the pH range 7—10. The observed K_h values decrease sigmoidally with pH as the thiol ionizes. Rearrangement of hemithioacetals formed from phenylglyoxal and various thiols was kinetically investigated. The rates increase with thiol concentration following a saturation curve to give K_h identical with the spectrophotometric value. The rearrangement is subject to general base catalysis. The solvent isotope effect on the rate of the rearrangement, k^{H_2O}/k^{D_2O}, is nearly 1.0; that on the equilibrium, $K_h^{H_2O}/K_h^{D_2O}$, is 0.38. The results strongly support the mechanism involving proton transfers through an enediol intermediate.

Glutathione (GSH), a tripeptide thiol, has been well known as a coenzyme which constitutes the glyoxalase enzyme system.[1] The enzyme catalyzes the Cannizzaro-type rearrangement of methylglyoxal to lactic acid. The suggestion that this action is related closely to the regulation of cell division[2] has stimulated interest in this enzyme system and its mechanism. The reaction proceeds through nonenzymatic formation of hemithioacetal and its rearrangement to α-hydroxy thiocarboxylic *S*-ester which is catalyzed by glyoxalase I:[1,3,4]



$$\mathrm{CH_3\text{-}\overset{O}{\overset{\|}{C}}\text{-}\overset{O}{\overset{\|}{C}}\text{-}H + GSH \rightleftharpoons CH_3\text{-}\overset{O}{\overset{\|}{C}}\text{-}\overset{OH}{\overset{|}{C}}H\text{-}SG}$$
$$\xrightarrow{\text{glyoxalase I}} \mathrm{CH_3\text{-}\overset{OH}{\overset{|}{C}}H\text{-}\overset{O}{\overset{\|}{C}}\text{-}SG}$$
$$\xrightarrow{\text{glyoxalase II}} \mathrm{CH_3\text{-}\overset{OH}{\overset{|}{C}}H\text{-}\overset{O}{\overset{\|}{C}}\text{-}OH + GSH.} \qquad (1)$$

Results of solvent isotope incorporation experiments led earlier investigators to conclude a mechanism involving an intramolecular hydride shift,[5,6] while recent NMR evidence of temperature-dependent incorporation of solvent protons suggested a mechanism of fast-shielded enediol proton transfer:[7]



$$\mathrm{R\text{-}\underset{O}{\underset{\|}{C}}\text{-}\underset{OH}{\underset{|}{C}}H\text{-}SG \longrightarrow R\text{-}\underset{OH}{\underset{|}{C}}{=}\underset{OH}{\underset{|}{C}}\text{-}SG}$$
$$\longrightarrow \mathrm{R\text{-}\overset{OH}{\overset{|}{C}}H\text{-}\overset{O}{\overset{\|}{C}}\text{-}SG.} \qquad (2)$$

Franzen[5,8] showed that certain 2-(dialkylamino)ethanethiols are efficient nonenzymatic catalysts for rearrangement of a glyoxal which mimicks the glyoxalase reaction. Hall and Poet[9] found that some α-keto hemithioacetals rearrange to α-hydroxy thiocarboxylic *S*-esters under catalysis of such bases as sodium acetate and tertiary amines in *N*,*N*-dimethylformamide solution. Shinkai *et al.*[10] have recently demonstrated the intermediacy of enediol in reactions of some thiols with methyl- and phenylglyoxals by the flavin-trapping experiments. However, the kinetic phase of this reaction has been examined only briefly[7b] and has not well been characterized.

In the present investigation, we examined reactions of various thiols with phenylglyoxal (PG) in aqueous solution. Equilibrium constants for formation of hemithioacetals were determined in a wide pH range and their rearrangement was kinetically characterized.

Experimental

Materials. Phenylglyoxal (PG) hydrate was prepared according to the literature.[11] 2-Mercaptoethanol (ME), 1-butanethiol, ethyl mercaptoacetate, and 3-mercaptopropionic acid were distilled and stored under nitrogen. Sodium mercaptoacetate (Wako Pure Chemicals) was used without further purification. Glutathione (GSH) was a gift from Ajinomoto Co. 1,4-Diazabicyclo[2.2.2]octane (DABCO) and imidazole were recrystallized from ligroine and benzene, respectively. *N*-Methylmorpholine, 1,1,1,3,3,3-hexafluoro-2-propanol, 2-(dimethylamino)ethanol, and *N*-methylpiperidine were distilled before use. The other inorganic chemicals were used as received. Glass-distilled water, which was freshly boiled under nitrogen and contained 10^{-4} M (1 M = 1 mol dm^{-3}) disodium dihydrogen ethylenediaminetetraacetate (EDTA), was used throughout. The ionic strength of solutions was always maintained with KCl.

Buffer Preparations. Buffer solutions for reaction were prepared immediately before use by mixing equal volumes of a stock buffer solution (μ=0.50) and a stock solution of thiol in 10% aqueous acetonitrile (μ=0.45). Concentrations of thiols were assayed by the Ellman method.[12,13] All the flasks for solution preparation and cuvettes for spectrophotometry were flushed with nitrogen. A Shimadzu UV 200 spectrophotometer was used for spectrophotometric determination. Measurements of pH of buffer and reaction solutions were carried out with a Hitachi-Horiba F-7 pH meter.[14]

Equilibrium Constants. Equilibrium constants K_h for hemithioacetal formation were determined by measuring increase in UV absorbance at 280 nm that occurs when PG is added to buffer solutions containing varying concentrations (0.001—0.01 M) of thiol at constant pH. The equilibrium was attained by addition of 0.030 cm^3 of aqueous solution of PG hydrate (1.3×10^{-2} M) to 3.0 cm^3 of the buffer solution which was equilibrated at 30±0.1 °C in a stoppered quartz cuvette inserted in a water-jacketed cell holder. The blank solution in the spectrophotometer was identical with the reaction solution, except that water was added in place of aliquot of the PG solution. Change in absorbance with time was followed until the absorbance remained constant at lower pH. Since at higher pH (>8) the absorbance decreased slowly after initial rapid increase, the reading was extrapolated to the time of PG addition. In some cases, measurements were carried out of absorbance decrease at 250 nm with $[\mathrm{PG}]_0 \simeq 7\times10^{-5}$ M.

Equilibrium constants K_h were calculated from absorbance change according to

$$K_h = \frac{[\text{hemithioacetal}]}{[\text{PG}][\text{RSH}]} = \frac{\Delta A}{(\Delta A_{\max} - \Delta A)[\text{RSH}]}, \tag{3}$$

where ΔA is the difference between the equilibrium absorbance of PG alone and that of PG plus thiol; $\Delta A_{\max}$ is a hypothetical absorbance change when all the PG is transformed to hemithioacetal.

$$\Delta A_{\max} = (\varepsilon_p - \varepsilon_h)[\text{PG}]_0, \tag{4}$$

where ε_p and ε_h are the extinction coefficients of PG and hemithioacetal, respectively. Equation 3 is transformed into

$$\frac{\Delta A}{[\text{RSH}]} = K_h \Delta A_{\max} - K_h \Delta A. \tag{5}$$

Plots of $\Delta A/[\text{RSH}]$ against ΔA (the Scatchard plot)[15,16] give $-K_h$ as a slope and $\Delta A_{\max}$ as an intercept of abscissa. Here, [RSH] is essentially equal to $[\text{RSH}]_0$ since $[\text{RSH}]_0 \gg [\text{PG}]_0$, but it was corrected for the hemithioacetal formation when necessary.

Kinetic Measurements. Rates of rearrangement were measured by following decrease in the absorption in the 280 nm region. The reaction was started in the same way as in the equilibrium measurement. Pseudo-first-order plots were usually linear over two lifetimes except for an initial short induction period due to the hemithioacetal formation. For slow reactions, a modified Guggenheim treatment[17] was employed.

Products of Reaction of 1-Butanethiol with PG. A sample of PG hydrate (0.5 g) was dissolved in 1 dm^3 of 0.1 M $(CF_3)_2CHOH$–0.1 M $(CF_3)_2CHONa$ buffer solution containing 0.005 M 1-butanethiol and 10 vol% acetonitrile (pH 9.4). After standing overnight at 30 °C the solution was neutralized and the volume was reduced to 100 cm^3 under reduced pressure. The remaining solution was acidified and extracted with ether. The extract was dried over $MgSO_4$ and the ether was evaporated. The residues were dissolved in D_2O. The ^{1}H NMR spectrum showed two singlet signals at 7.43 and 5.26 ppm from DSS in intensity ratio of 5:1, which is identical with the spectrum of an authentic sample of mandelic acid.

The reaction in D_2O–CH_3CN solution was carried out on a 100-cm^3 scale in the same way as above. Isotopic purity of the deuterium buffer solution was about 95%. The ^{1}H NMR spectrum of the products showed formation of mandelic acid but the signal of benzylidene α-hydrogen (5.26 ppm) was hardly perceived.

Results

Hemithioacetal Formation. Phenylglyoxal (PG) is almost completely hydrated in aqueous solution with absorption maximum at 250 nm (ε_{250} 11900, ε_{280} 1550). When PG hydrate is dissolved in a buffer solution containing an appropriate amount of thiol, the absorption spectrum changes rapidly with an isosbestic point at 267 nm due to formation of an adduct, hemithioacetal. The spectrum of the hemithioacetal had an absorption maximum at 250 nm and a shoulder at 280 nm. Extinction coefficients for the hemithioacetal of 2-mercaptoethanol (ME) were found to be $\varepsilon_{250}=9600$ and $\varepsilon_{280}=3150$ by extrapolation. Those for glutathione (GSH) were $\varepsilon_{250}=10100$ and $\varepsilon_{280}=3400$,

Table 1. Formation constants of hemithioacetals of PG[a)]

$HOCH_2CH_2SH$		GSH	
pH	K_h/M^{-1}	pH	K_h/M^{-1}
6.65	(752±73)[b)]	6.67	603±31 (625±71)[b)]
7.13	740±36	7.68	572±39
7.67	794±39	7.92	593±48
7.98	784±19	8.23	571±27
8.59	782±67	8.54	438±13
9.00	607±47	9.02	315±18
9.50	474±57	9.48	179±17
9.95	277±27	9.95	88±65
10.50	149±18		

a) Errors given are standard deviations. b) Measured at 250 nm.

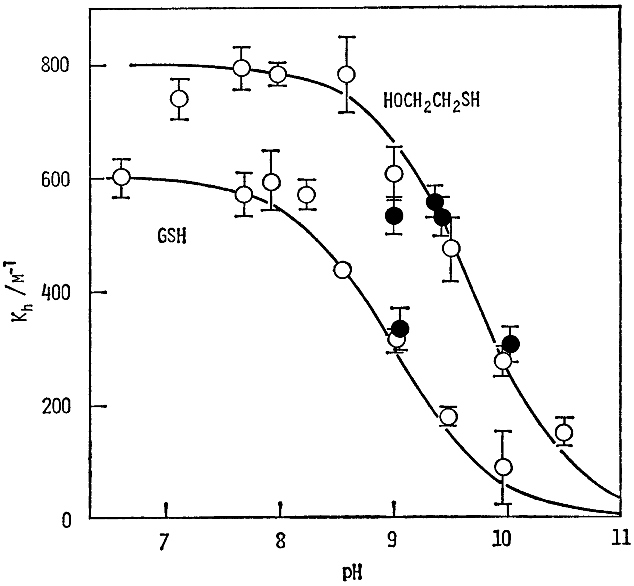
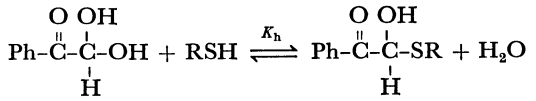


Fig. 1. pH dependence of the equilibrium constants K_h for hemithioacetal formation of ME and GSH. Closed circles are values obtained kinetically.



$$\text{Ph-}\overset{\text{O}}{\overset{\|}{\text{C}}}\text{-}\underset{\text{H}}{\underset{|}{\overset{\text{OH}}{\overset{|}{\text{C}}}}}\text{-OH} + \text{RSH} \overset{K_h}{\rightleftharpoons} \text{Ph-}\overset{\text{O}}{\overset{\|}{\text{C}}}\text{-}\underset{\text{H}}{\underset{|}{\overset{\text{OH}}{\overset{|}{\text{C}}}}}\text{-SR} + H_2O \tag{6}$$

Equilibrium constants for the hemithioacetal formation of PG with ME and GSH were determined by measuring increase in absorbance at 280 nm of PG of constant concentration in the presence of increasing concentration of thiol. Least-squares treatments of the Scatchard plots (Eq. 5) gave the equilibrium constants K_h summarized in Table 1. Values of K_h obtained from decreasing absorbance at 250 nm are included in Table 1. They agree well with those obtained at 280 nm.

The K_h values are not constant but seem to change sigmoidally with pH as shown in Fig. 1. The closed circles in the plots of Fig. 1 are the K_h values obtained kinetically as will be mentioned later. The sigmoid curves shown are those of pK_a=9.7 and 9.0

TABLE 2. RATES OF THE REARRANGEMENT OF HEMITHIOACETALS OF VARIOUS THIOLS[a)]

Thiol	pK_a [b)]	$10^3k_{obsd}/s^{-1}$ [c)]	$10^3k_{max}/s^{-1}$ [d)]	K_h/M^{-1} [d)]
$EtOCOCH_2SH$	7.95	1.78	3.75±0.70	187± 55[e)]
GSH	8.7	3.10	4.96±0.50	331± 46
$HO(CH_2)_2SH$	9.7	1.29	1.80±0.06	533± 35
$DO(CH_2)_2SD$[f)]		1.60	1.89±0.11	1410±159
n-BuSH	10.7	(1.40)[g)]	1.69±0.14	1020± 80
$^-OCOCH_2SH$	10.7	1.11	1.28±0.30	1300±210
$^-OCO(CH_2)_2SH$	10.8	1.12		

a) 5% CH_3CN-H_2O ($\mu=0.475$); [DABCO]=0.2 M; pH 9.0±0.5; 30 °C. b) pK_a of thiol taken from "CRC Handbook of Biochemistry," ed by H. A. Sober, Chem. Rubber Co., Cleveland (1968). c) k_{obsd} at [RSH]=0.005 M. d) Errors given are standard deviations. e) Value 1000±44 M^{-1} at pH 5.8 was obtained spectrophotometrically. f) 5% CH_3CN-D_2O; pD 9.74. g) Calculated value.

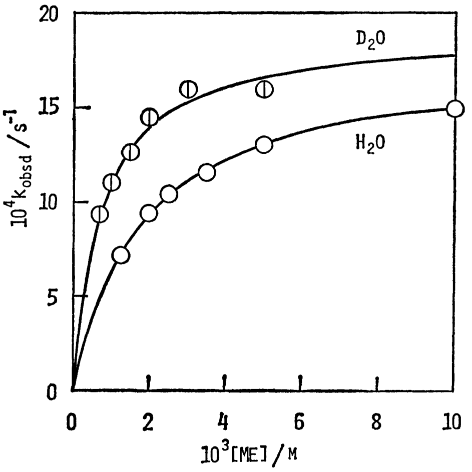
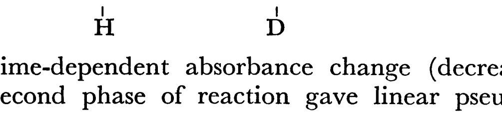


Fig. 2. Dependence of rates of the rearrangement of hemithioacetal upon thiol concentrations in 5% CH_3CN-H_2O (O) and 5% CH_3CN-D_2O (◐) at 30 °C. $[DABCO]_t=0.2$ M, buffer ratio=1, $\mu=0.475$ (KCl).

for ME and GSH, respectively. Ultimate K_h values at lower pH were taken to be 800 and 600 M^{-1} for ME and GSH, respectively, and those at higher pH to be zero.

Rearrangement. On addition of PG in a thiol-containing buffer solution, the absorbance in the 280 nm region decreases slowly after the initial rapid increase due to the formation of hemithioacetal. The slow decrease in absorbance is ascribed to rearrangement of the hemithioacetal to an α-hydroxy thiocarboxylic *S*-ester. The thioester is further hydrolyzed to mandelic acid in much smaller rate.[18)] The ultimate product obtained in the reaction with 1-butanethiol was identified by ^1H NMR spectroscopy. The product obtained from the reaction in deuterium media (D_2O-CH_3CN) showed incorporation of deuterium at the benzylidene α-position:



$$\text{Ph-}\overset{\text{O}}{\overset{\|}{\text{C}}}\text{-}\underset{\text{H}}{\overset{\text{OD}}{\text{C}}}\text{-SR} \xrightarrow{D_2O} \text{Ph-}\underset{\text{D}}{\overset{\text{OD}}{\text{C}}}\text{—}\overset{\text{O}}{\overset{\|}{\text{C}}}\text{-SR}. \quad (7)$$

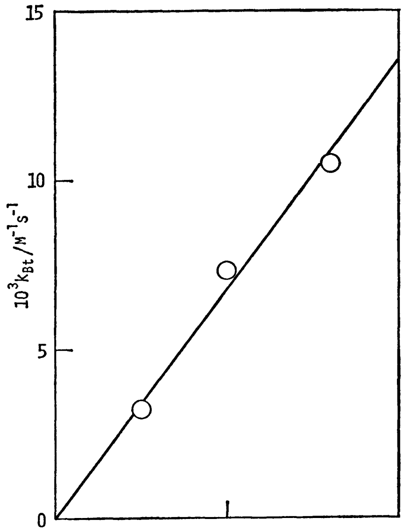


Fig. 3. Buffer-dependent rate constants k_{Bt} *vs.* base fraction of DABCO buffers. $[ME]_0=0.05$ M, 5% CH_3CN-H_2O ($\mu=0.475$), 30 °C.

The time-dependent absorbance change (decrease) in the second phase of reaction gave linear pseudo-first-order plots, from which rates of the rearrangement were obtained. The observed rate constants k_{obsd} showed saturation with increasing thiol concentration [RSH] as shown in Fig. 2 in accord with the rate equation

$$k_{obsd} = \frac{k_{max}K_h\,[RSH]}{1+K_h\,[RSH]}. \quad (8)$$

Parameters k_{max} and K_h were determined from linear plots of the Scatchard-type (Eq. 9) or double reciprocal plots (Eq. 10):

$$\frac{k_{obsd}}{[RSH]} = K_hk_{max} + K_hk_{obsd}, \quad (9)$$

$$\frac{1}{k_{obsd}} = \frac{1}{k_{max}} + \frac{1}{k_{max}K_h}\frac{1}{[RSH]}. \quad (10)$$

Kinetic parameters k_{max} and K_h in a DABCO buffer of pH 9 are summarized in Table 2 for various thiols. Results in D_2O are also included. The equilibrium constants K_h obtained here agree very well with those obtained spectrophotometrically for the formation of hemithioacetal as illustrated in Fig. 1.

At constant [RSH], rates increase linearly with buffer concentration:

TABLE 3. RATE CONSTANTS FOR THE REACTION OF PG AND ME IN VARIOUS BUFFER SOLUTIONS[a)]

pH	Buffer	$\frac{10^4k_0}{\mathrm{s}^{-1}}$ b)	$\frac{10^3k_{\mathrm{Bt}}}{\mathrm{M}^{-1}\,\mathrm{s}^{-1}}$ b)	$\frac{10^3k_{\mathrm{B}}}{\mathrm{M}^{-1}\,\mathrm{s}^{-1}}$ b,c)	$\frac{10^3k_{\max}}{\mathrm{s}^{-1}}$ d)	$\frac{K_{\mathrm{h}}}{\mathrm{M}^{-1}}$ d)
8.30	*N*-Methylmolpholine	0.21	0.337	0.45		
8.48	DABCO	1.02	3.20			
9.00	DABCO	1.62	7.48	13.5	1.80 ±0.06	533±35
9.36	$(CF_3)_2CHOH$	(4.0)	(6.83)	(13.7)	1.71 ±0.04	558±28
9.48	$(CH_3)_2N(CH_2)_2OH$	3.10	2.90	5.8	0.752±0.072	529±31
9.62	DABCO	7.42	10.5			
10.03	Carbonate	(6.5)	(10.6)	(21.2)	2.67 ±0.23	306±30
10.32	*N*-Methylpiperidine	16.3	10.7	21.4		

a) In 5% CH_3CN-H_2O ($\mu=0.475$) at 30 °C. b) [ME]=0.05 M. Values in parentheses are those calculated from data of buffer dependence at [ME]=0.005 M. c) Estimated from k_{Bt} for OH^- is estimated to be 8.0 $\mathrm{M}^{-1}\,\mathrm{s}^{-1}$ from k_0 values. d) $[B]_t=0.2$ M. Errors given are standard deviations.

$$k_{\mathrm{obsd}} = k_0' + k_{\mathrm{Bt}}'[\mathrm{B}]_t \tag{11}$$

or

$$k_{\max} = k_0 + k_{\mathrm{Bt}}[\mathrm{B}]_t. \tag{12}$$

Since $k_{\mathrm{obsd}} \simeq k_{\max}$ at $[\mathrm{RSH}] \gg 1/K_{\mathrm{h}}$, k_0, and k_{Bt} can be evaluated from buffer-dependence data obtained by keeping [RSH] large enough and constant. Table 3 summarizes kinetic results for the reaction of ME with PG under various conditions. Constants k_0 and k_{Bt} were obtained mostly by keeping [RSH]=0.05 M. Buffer-dependent rate constants k_{Bt} increase with increasing base fraction of the buffer (Fig. 3); the reaction is catalyzed by general bases. Catalytic constants k_{B} of some general bases estimated from results of one buffer ratio are included in Table 3.

Discussion

Reaction of thiols with aldehydes to form hemitioacetals has been well-known and some hemithioacetals of α-keto aldehydes have been even isolated.[19)] However, equilibrium and kinetic studies are limited only in a few cases.[20—23)] Equilibrium constants for the formation of hemithioacetals from GSH and various α-keto aldehydes were measured in relation to the glyoxalase reaction.[23)] Values K_{h} were found to be essentially constant in the pH range 5—9.

The present results clearly show that K_{h} values sharply change in the pH range 8—10 for GSH and 9—11 for ME. The changes seem to follow sigmoid curves of $\mathrm{p}K_{\mathrm{a}}=9.0$ and 9.7 for GSH and ME, respectively. These $\mathrm{p}K_{\mathrm{a}}$ values correspond to those of the respective thiols; that is, only the neutral form of the thiol contributes to the formation of hemithioacetal at equilibrium but the thiolate ion is hardly effective. This is reasonable when we consider an equilibrium cycle:

$$\begin{array}{ccc} \mathrm{RSH} + \mathrm{R'CH(OH)_2} & \overset{K_{\mathrm{h}}}{\rightleftharpoons} & \mathrm{R'CH}\!\begin{smallmatrix}\diagup\mathrm{OH}\\ \diagdown\mathrm{SR}\end{smallmatrix} + \mathrm{H_2O} \\ K_{\mathrm{a}}\Big\updownarrow & & K_{\mathrm{a}}'\Big\updownarrow \\ \mathrm{RS^-} + \mathrm{R'CH(OH)_2} & \overset{K_{\mathrm{h}}'}{\rightleftharpoons} & \mathrm{R'CH}\!\begin{smallmatrix}\diagup\mathrm{O^-}\\ \diagdown\mathrm{SR}\end{smallmatrix} + \mathrm{H_2O}. \end{array} \tag{13}$$

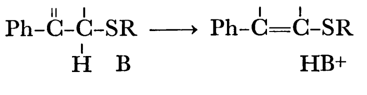
The ratio $K_{\mathrm{h}}/K_{\mathrm{h}}'$ should be equal to $K_{\mathrm{a}}/K_{\mathrm{a}}'$ which must be greater than 10^2.[24)]

Rates of the rearrangement are dependent on [RSH] with saturation as seen in Fig. 2. Analysis of the curve gives the equilibrium constant K_{h} and the maximum rate constant $k_{\max}$. The K_{h} values agree very well with those obtained independently by the spectrophotometric method. This observation firmly indicates that the hemithioacetal is a true intermediate of the reaction.

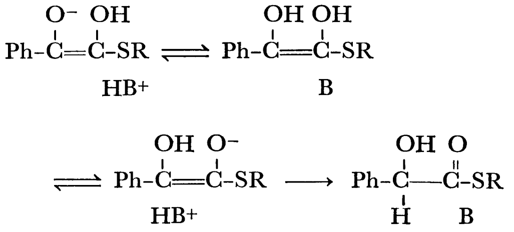
The rate constants $k_{\max}$ correspond to those of the rearrangement when all the PG is transformed into the hemithioacetal. The $k_{\max}$ values increase with buffer concentrations and the buffer-dependent rate constants k_{Bt} were found to be ascribed to a base form of the buffer (Fig. 3); the reaction is confirmed to be catalyzed by general bases.[26)] The catalytic constants k_{B} summarized in Table 3 are not precise enough for further analysis but Brønsted β of about 0.45 may be estimated including $k_{\mathrm{OH^-}}$ value of 8 $\mathrm{M}^{-1}\,\mathrm{s}^{-1}$. The latter value involves another uncertainty owing to the k_0 values influenced by the term of general-base catalysis due to 0.05 M ME.

General base catalysis established here is consistent with a mechanism involving the enediol proton transfer.[7)] This mechanism was concluded from the observation that the solvent proton is incorporated into the product for the glyoxalase reaction[7a)] as well as a model reaction with 2-(diethylamino)ethanethiol.[7b)] Similar results were obtained in the present reaction with a simple thiol. The product mandelic acid formed by the reaction of 1-butanethiol with PG in D_2O was found to have lost a proton at the benzylidene α-position.

Solvent isotope effects observed in a DABCO buffer are $k_{\max}^{\mathrm{H_2O}}/k_{\max}^{\mathrm{D_2O}}=0.95\pm0.09$ and $K_{\mathrm{h}}^{\mathrm{H_2O}}/K_{\mathrm{h}}^{\mathrm{D_2O}}=0.38\pm0.07$. The rate constant $k_{\max}$ is little affected by solvent isotopes.



$$\underset{\mathrm{H}\quad\mathrm{B}}{\mathrm{Ph\text{-}}\overset{\mathrm{O}}{\overset{\|}{\mathrm{C}}}\text{-}\overset{\mathrm{OH}}{\overset{|}{\mathrm{C}}}\mathrm{\text{-}SR}} \longrightarrow \underset{\mathrm{HB^+}}{\mathrm{Ph\text{-}}\overset{\mathrm{O^-}}{\overset{|}{\mathrm{C}}}=\overset{\mathrm{OH}}{\overset{|}{\mathrm{C}}}\mathrm{\text{-}SR}} \tag{14}$$



$$\underset{\mathrm{HB^+}}{\mathrm{Ph\text{-}}\overset{\mathrm{O^-}}{\overset{|}{\mathrm{C}}}=\overset{\mathrm{OH}}{\overset{|}{\mathrm{C}}}\mathrm{\text{-}SR}} \rightleftharpoons \underset{\mathrm{B}}{\mathrm{Ph\text{-}}\overset{\mathrm{OH}}{\overset{|}{\mathrm{C}}}=\overset{\mathrm{OH}}{\overset{|}{\mathrm{C}}}\mathrm{\text{-}SR}}$$

$$\rightleftharpoons \underset{\mathrm{HB^+}}{\mathrm{Ph\text{-}}\overset{\mathrm{OH}}{\overset{|}{\mathrm{C}}}=\overset{\mathrm{O^-}}{\overset{|}{\mathrm{C}}}\mathrm{\text{-}SR}} \longrightarrow \underset{\mathrm{H}\qquad\mathrm{B}}{\mathrm{Ph\text{-}}\overset{\mathrm{OH}}{\overset{|}{\mathrm{C}}}\text{—}\overset{\mathrm{O}}{\overset{\|}{\mathrm{C}}}\mathrm{\text{-}SR}} \tag{15}$$

The rate-determining step must be proton transfer from the hemithioacetal to the base (DABCO) to form an enediol (Eq. 14). Protonation of the enediol at the β position (Eq. 15) should be faster than that at the α position (reverse of Eq. 14). The primary isotope effect of $k_H/k_D=2.3$ observed with PhCOCDO[7b)] is also in accord with this mechanism. Inverse solvent isotope effects on the equilibrium constant K_h are not unreasonable on the basis of the isotopic fractionation factor of about 0.4—0.5 of thiol hydrogen.[27,28)] Similar isotope effects of $K_h^{H_2O}/K_h^{D_2O}=0.44$ were found for the formation of hemithioacetal of acetaldehyde.[20a)]

Kinetic parameters summarized in Table 2 were obtained with various thiols at pH 9 in DABCO buffers. Rates of rearrangement (k_{max}) show a tendency to decrease with increasing pK_a of the thiol. This is consistent with the mechanism involving the rate-determining deprotonation to form the enediol intermediate. Electron-withdrawing effects which lower pK_a of the thiol may ease the deprotonation of the hemithioacetal. Effective catalytic function of an intramolecular general base group of the thiol was found with *N*-(2-mercaptoethyl)piperidine and -morpholine in aqueous solution as will be reported in a separate paper.[29)] However, the carboxylato group of mercaptoacetate and 3-mercaptopropionate does not seem to show any effective catalytic activity. Enhanced reactivity of GSH might be due partly to such intramolecular effects of the amino group. Shinkai *et al.*[10)] noted low catalytic efficiency of GSH contrary to our observation. This is probably due to their use of borate buffers. Borate forms adducts with PG in aqueous solution[29)] to retard the rearrangement. Furthermore, GSH itself may form some adduct with boric acid.

Magnitudes of K_h depend largely on the degree of ionization of the thiol in such a way that the ionization results in small value of K_h, but the thiol structure shows minor influence on the K_h value. As a whole, the catalytic efficiency of simple thiols in the rearrangement is not largely dependent on their structure unless the thiol is ionized under the reaction conditions. All the rearrangement reactions of α-keto hemithioacetals proceed through proton transfers involving the enediol as an intermediate.

We thank Mr. H. Nagamatsu for his assistance in kinetic measurements.

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