MECHANISM OF THE REACTION OF THIOLS WITH SELENITE

by

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INTRODUCTION

Although only a trace element, in recent years selenium has been shown to have very important physiological effects.

Over the past decade, nutritional research has shown that selenium is intimately involved, together with vitamin E, in the prevention of a number of serious deficiency diseases in various species of livestock and poultry. Proof of the specific nutritional essentiality of selenium was obtained by Thompson and Scott[1], who showed that chicks and Japanese quail receiving diets containing synthetic amino acids and other purified ingredients extremely low in selenium fail to grow, and suffer high mortality unless their diet is supplemented with selenium. Addition of all known nutrients, including high levels of vitamin E, failed to prevent severe deficiency signs and death, whereas addition to the basal high-vitamin E diet of as little as 0.2 ppm of selenium (as sodium selenite) completely prevented all deficiency signs and promoted a good rate of growth in the chicks and quails. Various diseases have also been reported[2] as originating from a deficiency of selenium, such as necrotic liver degeneration in rats, mices, rabbits, and pigs; muscular dystrophies (myopathies) in lambs; "unthriftness" in cattle and sheep; poor hair development in

pigs and horses; and poor feather development in chickens; exudative diathesis in chicks; and atrophy and fibrosis of the pancreas in chicks. All these diseases can also be prevented by supplementing a small quantity of selenium in the diet.

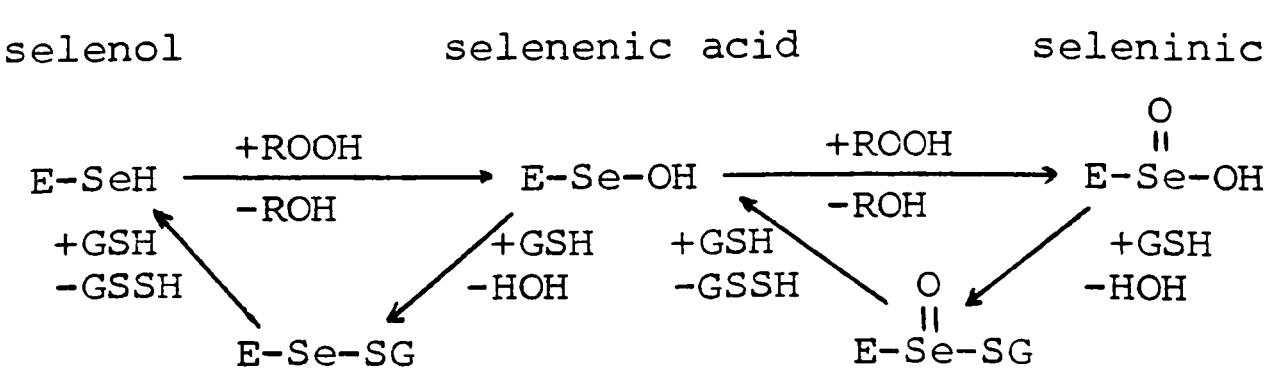
Although selenium has now been shown to be required entirely apart from its interaction with vitamin E, the action of its prevention of deficiency diseases in animals is very intimately connected with that of vitamin E. Studies[3] have indicated that vitamin E may be carried by a seleno-lipoprotein fraction associated with serum γ-globulin. Thus one biological role of selenium appears to lie in a selenium-containing compound which acts as a carrier of vitamin E and which may function in the absorption, retention and prevention of destruction of d-α-tocopherol, and perhaps in its transfer across cell membranes, thereby enhancing its biological activity in the blood and perhaps in the cells throughout the body.

On the other hand, the ingestion of seleniferous plants by livestock, with the consequent development of well-defined disorders, blind staggers and alkali dieseases, has been a problem to stockmen and farmers for centuried. These ambidextrous effects of selenium have led to the investigation of the role it plays in metabolism. Unfortunately, there is still little known about it.

However, in 1975, Ganther[4] found that glutathione peroxidase isolated from erythrocytes and liver contains 4 gram atoms of selenium in a tetrameric complex of 84,000-95,000 molecular weight. This discovery means that selenium is an integral, stoichiometric component of a protein in a living system. The experimental evidence supporting the direct participation of selenium in the reaction comes from X-ray photoelectron spectroscopic studies, which suggest that oxygen is combined with the selenium in the oxidized form, but not in the reduced enzyme. This supports a general mechanism in which selenium undergoes a 2-electron oxidation-reduction reaction, (Eq. 1).

$$R\text{-}Se(O)_nH \underset{+2GSH,\ -GSSG,\ -HOH}{\overset{+ROOH,\ -ROH}{\rightleftharpoons}} R\text{-}Se(O)_{n+1}H \qquad (1)$$

Based on this fact, and the kinetic studies of Flohe's group[5,6], a simple, tentative mechanism for glutathione peroxidase involving a series of three bimolecular reactions has been proposed by Ganther[4]; it is shown in Scheme I.



Scheme I

The first step is oxidation of an active site selenol (E-SeH) to a selenenic acid (E-Se-OH) by peroxide substrate. In the second step, the selenenic acid reacts with the first glutathione (GSH), forming the sulfoselenide (E-Se-SG) and a molecule of water. In the final step, the sulfoselenide linkage is cleaved by a second molecule of glutathione, similar to a sulfhydryl-disulfide interchange reaction, releasing oxidized glutathione and restoring the enzyme to the selenol form. Ganther has claimed that the sulfoselenide (R-S-Se-R) type of linkage is cleaved by thiols even more readily than the disulfide linkage. However, other workers[7-10] have reported results that cast some doubt upon whether or not this is necessarily true. An analogous set of reactions can be written in which the selenium cycles between the selenenic (E-SeOH) and the seleninic ($E-SeO_2H$) forms. It is possible that both types of mechanism could operate, depending on the relative concentrations of oxidizing and reducing substrates.

With these facts in mind, we can conclude that selenium plays an important role in living systems. Given that selenium is an important trace element in metabolism, how does it get incorporated into animals and plants initially?

Most studies on the metabolic incorporation of selenium have involved the use of inorganic selenium (i.e.

selenite and selenate). Although *in vitro* studies have indicated that mammalian tissues are capable of reducing selenite to the red allotrope of elemental selenium[11,12], there is no evidence at present indicating that such a reduction occurs *in vivo*[13]. Ganther has presented excellent evidence[14] showing that selenite combines with biologically important sulfhydryl compounds such as cysteine, glutathione, and coenzyme A to form "selenotrisulfide" derivatives (RS-Se-SR). The combining ratio for the thiols and selenious acid was found to be 4:1. The overall stoichiometry thus conforms to the reaction proposed by Painter[15] in 1941 (Eq. 2):

$$4\ RSH + H_2SeO_3 \longrightarrow RSSeSR + RSSR + 3\ H_2O \qquad (2)$$

He has proposed that this reaction is probably how the selenium in selenite gets incorporated into plants or animals in biological systems. The toxicity of selenite might be due to its ability to catalyze the oxidation of important sulfhydryl cofactors such as gluthione, coenzyme A, etc., thus resulting in disturbances in intermediary metabolism.

Ganther and Corcoran[16] have presented spectral evidence showing that reduced ribonuclease combines with selenite, forming a selenotrisulfide type of product. Before proteins can react with selenite to form selenotrisulfides,

the protein would necessarily have to exist as free sulfhydryls. At the completion of protein synthesis, the sulfhydryls of most proteins are largely converted to disulfide groups. Indeed, it was found that reduced ribonuclease and not the native protein reacted with selenite to form a selenotrisulfide. Jenkins et. al.[17] reported that selenite added to chick serum in vitro does not complex with the protein. This observation led to the postulate that selenium is incorporated into proteins during the final stages of protein synthesis.

In a separate investigation Jenkins[18] crop-incubated one chick with $Na_2{}^{75}SeO_3$ and another chick with a ^{14}C-labeled L-amino acid mixture. Serum proteins from both chicks were then dialyzed for 17 hours at pH 11.5. Such alkaline treatment brought about a release of one-third of the ^{75}Se activity, but no such release was observed in the serum of the chick administered the ^{14}C-labeled amino acid mixture. This observation indicated that the release of selenium from the protein was not the result of protein hydrolysis, thus negating the possibility that peptide-bound seleno-amino acids had been released. Furthermore, Jenkins was unable to identify either selenocystine or selenomethionine in the alkaline dialysate.

In this investigation[18] and a subsequent one[17] from

Jenkins laboratory it was postulated that the selenium was bound to the protein through a selenotrisulfide type of bond similar to that proposed by Painter. Jenkins[18] then postulated a series of equations (Scheme II) illustrating how the protein-selenotrisulfide complex could dissociate under alkaline conditions to reform selenite, thus accounting for the release of selenium from proteins under alkaline conditions.

$$2\ PS\text{-}Se\text{-}SP + 2\ OH^- \longrightarrow 2\ PS^- + 2\ PS\text{-}SeOH$$

$$2\ PS\text{-}SeOH \longrightarrow PSH + PS\text{-}SeO_2H + Se$$

$$PS\text{-}SeO_2H + 2\ OH^- \longrightarrow PSH + SeO_3^{-2} + H_2O$$

$$2\ PS\text{-}Se\text{-}SP + 6\ OH^- \longrightarrow 4\ PS^- + Se + SeO_3^{-2} + 3\ H_2O$$

$$2\ PS\text{-}Se\text{-}SP + 4\ OH^- \longrightarrow 2\ PS^- + Se + SeO_3^{-2} + H_2O$$

(P = protein moiety)

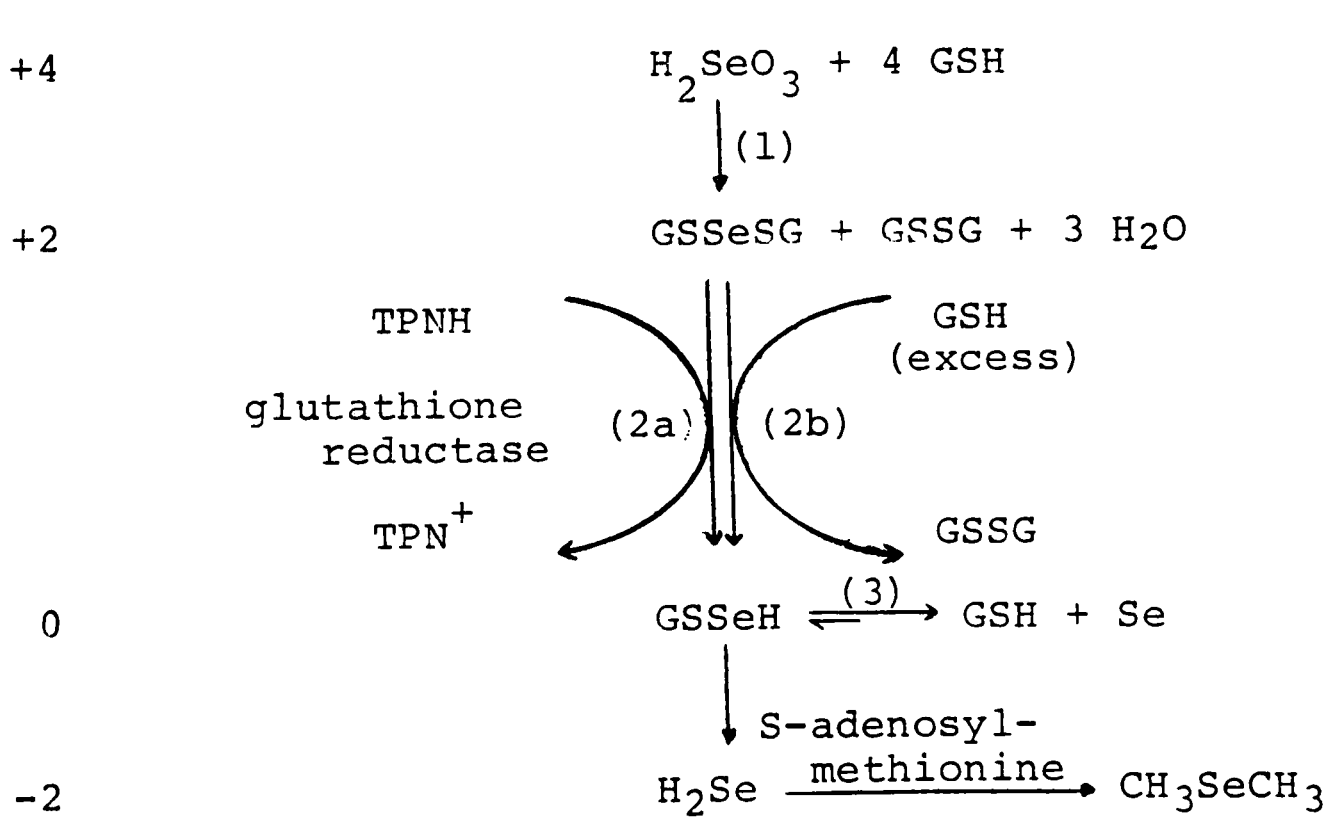
Scheme II

Recently, Ganther[19] has found that the selenotrisulfide derivative of glutathione can be reduced to a selenopersulfide either by the excess glutathione (Eq. 3) or by a glutathione reductase (Eq. 4).

$$GSSeSG + GSH \longrightarrow GSSeH + GSSG \qquad (3)$$

$$GSSeSG + TPNH + H^+ \xrightarrow{\text{glutathione reductase}} GSH + GSSeH + TPN^+ \qquad (4)$$

In either case, a TPNH-linked process requiring glutathione reductase is ultimately involved in the reduction of selenium *in vivo* to GSSeH. This labile, highly reactive selenopersulfide derivative of glutathione probably has a role in selenium metabolism and possibly in certain biological functions of selenium. Ganther has proposed a pathway (Scheme III) for the synthesis of dimethylselenide, which is a detoxification product of selenite in animals, in which GSSeH has a central role.



Scheme III

Reactions (1)-(3) in the diagram are established with rea-

sonable certainty. The further metabolism of selenium to the -2 oxidation state, with ultimate methylation, though the pathway has not been established, is known to occur.

In view of the great physiological importance of selenium, obviously more detailed information regarding the exact mechanism of the thiol-selenite reaction is vitally important. However, up until the time that the present work was begun there had been no mechanistic studies on equation (1) and nothing was known about its mechanism. This was the reason for the present study.

In preliminary work on thiol-selenite reaction in this laboratory, Dr. Thomas W. S. Lee followed the reaction of aqueous solution of selenious acid with 1-butanethiol in 60% dioxane. Study over a pH range from 0.9 to 10 showed that the reaction took place in two distinct stages, a rapid initial stage followed by a much slower second stage. Kinetic study of the first stage by Lee showed that the reaction has a first-order dependence on thiol concentration. The pH-rate profile associated with the first stage showed that the only process of kinetic importance was reaction of 1-butanethiol with selenious acid.

In one part of the present study, the kinetic behavior of the second stage of the reaction of 1-butanethiol with selenious acid was investigated in detail. In a second part of the present study, the detailed kinetics and other beha-

vior of both the first and second stages of the reaction of 2-methyl-2-propanethiol with selenious acid were studied. Comparison of the behavior of the two stages of the reaction of this tertiary thiol with H_2SeO_3 with the behavior exhibited by the analogous stages of the reaction of the primary thiol (n-BuSH) with H_2SeO_3 has enabled one to draw a number of further significant conclusions about the mechanism of each of the stages of the thiol-selenite reaction.

Both the products of the n-BuSH-H_2SeO_3 reaction and the t-BuSH-H_2SeO_3 reaction have also been studied. It was found that, besides the selenotrisulfide, RSSeSR, and the disulfide, RSSR, thiolsulfonates, $RS(O)_2SR$, n-butyl 1-butanethiolsulfonate, and t-butyl 2-methyl-2-propanethiolsulfonate were also formed. Although the thiolsulfonate is a very minor product in the case of the n-butyl mercaptan reaction it is a major product in the case of the reaction of t-BuSH with selenite. The significance of this and other observations regarding the products of the thiol-selenite reaction as compared with those of the reaction of thiol with benzeneseleninic acid are discussed.

It is known that a stable intermediate of structure PhSe(O)SBu-t can be isolated by lyophilization in the reaction between benzeneseleninic acid and 2-methyl-2-propanethiol[20]. The same method was also applied to the reaction

between selenious acid and 2-methyl-2-propanethiol to try to isolate an intermediate at the end of the second stage of the reaction. This attempt was never successful. Either the intermediate is not very stable and readily reacts further with more thiol molecules to form the final products or else it decomposes during the stage of lyophilization. However, this is consistent with the results found in the product studies that indicate that the last stages of thiol-selenite reaction proceeds via a different mechanism than the reaction of a thiol with PhSe(O)Bu-t.

Combining all of the kinetic studies with the studies of products and the search for an intermediate allows one to determine the mechanism of reaction between thiol and selenious acid with reasonable certainty. The present thesis describes these studies in detail and what conclusions the results lead to.

RESULTS

Both the mercaptans studied in this work, 1-butanethiol, n-BuSH, and 2-methyl-2-propanethiol, t-BuSH, and selenious acid are transparent in ultraviolet region down to 230 nm. This makes it easy to follow the reaction of either thiol with selenite (Eq. 2) spectrophotometrically. When an aqueous solution of selenious acid was treated with 1-butanethiol in 60% dioxane (v/v), an absorption shoulder at 248-262 nm appeared rapidly (Figure 1, Spectrum a). This absorption then decreased slowly accompanied by a large increase in the absorption at 230 nm and formation of a broad band ranging from 260 nm out to 380 nm. There was an isobestic point at 248 nm. The final spectrum of the solution was found to be very similar to that reported by Ganther[14] for a selenotrisulfide, which in this case would be the n-butyl compound, n-BuSSeSBu-n (Figure 1, Spectrum b).

When the same reaction was carried out in 0.13 M $HClO_4$ in 60% dioxane, the rate of the initial reaction was extremely fast and the shape of the absorption in the 248-262 nm region was somewhat different (Figure 2, Spectrum a), but in the slower second stage there was again a large increase in absorbance at 230 nm and formation of a broad band at 260-360 nm (Figure 2, Spectrum b).

The reaction was also carried out in 60% dioxane containing various buffers. In formate buffer (pH = 6.5), however, in the initial stage a well-developed absorption maximum developed at 262 nm (Figure 3, Spectrum a). Then, within hours, this peak decreased in absorbance with the appearance of strong absorbance at 230 nm and a broad band at 260-360 nm (Figure 3, Spectrum f).

The instaneous absorption around 262 nm presumably corresponds to the formation of the intermediate that is the product of the first stage of the reaction of 1-butanethiol with selenious acid. The broad band ranging from 260 nm to 380 nm that develops during the second kinetic stage of the reaction could either be due to the build-up of a second intermediate or to the formation of the final selenotrisulfide product (Eq. 5).

$$H_2SeO_3 \xrightarrow[RSH]{k_a} \text{Intermediate I} \xrightarrow[RSH]{k_b} \text{Intermediate II} \xrightarrow[2RSH]{} RSSeSR \quad (5)$$

With t-BuSH as the reacting thiol the rate of the second stage is at least 250 times slower than the rate of the first stage at all pH's studied, so that one has essentially complete conversion of selenious acid to the 262 nm intermediate before the conversion of this intermediate to subsequent intermediates or final products becomes significant. Thus, the kinetics of the two stages can be easily investigated separately. Repetitive scanning of the spectrum shows

that the best wavelength to use for study of the second stage of the reaction is 310 nm, since the difference of extinction coefficients between intermediate I and II (or final products) is greatest at this point (Figure 3). The kinetics of the first stage are best followed at 262 nm where there is a distinct absorption peak. But two wavelengths other than 262 nm, 253 and 273 nm, have also been used to follow the first stage, depending on which of them provides the best isobestic point for intermediate I and the later intermediates (or final products) in different buffers.

On the other hand, when the thiol is n-BuSH the situation is a little different. At most pH's (up to 6.5), the rate of the first stage is still faster (at least 16 times) than that of the second stage, so that the rates of the two stages still can be easily measured independently. The first stage is followed either at 262 nm or 248 nm, while the second stage is followed at 310 nm. However, in acetate buffers (pH = 7.4 and pH = 8.1) the rates of the two stages are comparable. That is, intermediate I begins to react further with another molecule of 1-butanethiol to give intermediate II before its formation from n-BuSH and selenious acid is complete, thus preventing one from getting accurate rates for the first stage by following the reaction at 262 nm. Fortunately, 248 nm is still an isobestic point for intermediate I and later intermediates for reaction of n-BuSH with

selenite in these buffers and accurate rates can thus be measured at this wavelength.

Kinetics of the Second Stage of the Reaction of 1-Butanethiol with Selenious Acid

The kinetics of the second stage of the reaction of 1-butanethiol, n-BuSH, with H_2SeO_3 were studied spectrophotometrically at 25°C in 60% dioxane under conditions where the thiol was always present in large stoichiometric excess over the selenious acid. The variation of rate with pH was examined over a range extending from 0.6 M $HClO_4$ (pH = 0.2) to an acetate buffer having a pH of 8.1 in 60% dioxane. The rates for the second stage under all these conditions were obtained by monitoring the change in optical density with time at 310 nm. As noted in the preceding section the reaction of the thiol with selenious acid is kinetically biphasic, with the rate of the first stage being enough faster than the rate of the second that one can measure the rates of the second stage without interference from the first stage. The variation of the rate with mercaptan concentration showed that this stage, conversion of intermediate I to intermediate II (step k_b), was first order in mercaptan. The rate data for the second stage of the n-BuSH-H_2SeO_3 reaction at various pH's are collected in Table I.

Figure 4 shows the pH-rate profile for the second stage of the n-BuSH-H_2SeO_3 reaction. The pH's of the various car-

boxylate buffers in 60% dioxane were derived from the measured pH's for each buffer in water and previous measurements, either in this laboratory[21], or others[22], of pK_a for each of the carboxylic acids for transfer from water 60% dioxane as solvent. The pH-rate profile is somewhat U-shaped. The rate decreases rapidly with increasing pH from pH = 0.2 to pH = 2; it then levels off within the range of pH = 2 to pH = 4; after that it increases with increasing pH until pH = 6.5 where it begins to level off again. The significance of the pH-rate profile will be discussed later.

Kinetics of the First Stage of the Reaction of 2-Methyl-2-propanethiol with Selenious Acid

The kinetic studies of the first stage of the t-BuSH-H_2SeO_3 reaction were first carried out in formate buffer (pH = 6.5) by following the absorbance increase with time at 262 nm. It was found that the absorbance change, $A_\infty - A_o$, varied with the concentration of 2-methyl-2-propanethiol. The absorbance change, $A_\infty - A_o$, first increased with increasing concentration of 2-methyl-2-propanethiol, then leveled off when the thiol concentration reached considerably higher values (0.04 M). Furthermore, the kinetic studies showed that the rate constant of the pseudo first order reaction, k_i, was not just simply proportional to the concentration of 2-methyl-2-propanethiol. Rather it followed the relationship:

$$k_i = k_o + k_a[t\text{-BuSH}] \qquad (6)$$

when k_i's were plotted vs. the concentrations of the thiol. These constants k_i, k_o, and k_a are summarized in Table II.

Both the above facts indicate that the first stage of this reaction is an equilibrium (Eq. 7).

$$H_2SeO_3 + \text{t-BuSH} \underset{k_o}{\overset{k_a}{\rightleftharpoons}} \text{Intermediate I} \qquad (7)$$

Since the second stage is proceeding much slower than the first stage for this reaction, it is possible to estimate the equilibtium constant K_{eq} by measuring the variation of absorbance change, $A_\infty - A_o$, versus the concentration of 2-methyl-2-propanethiol. The equilibrium constant estimated by this method agrees reasonably well with the equilibrium constant K_{eq} that is estimated from the kinetic studies, by assuming that K_{eq} is given by the ratio k_a/k_o.

The pH-rate profile for k_a of the first stage of t-BuSH-H_2SeO_3reaction is shown in Figure 6. This profile is essentially similar to that for the first stage of n-BuSH-H_2SeO_3 reaction[23] (Figure 5). The rate remains nearly constant in the region from pH = 0.2 up to pH = 4.5, then decreases rapidly with increasing p from pH = 4.5 to pH = 7.4. This suggests that the first stage of both reactions is proceeding by the same mechanism, except their reaction rates are different.

Kinetics of the Second Stage of the Reaction of 2-Methyl-2-propanethiol with Selenious Acid

As already noted, the second stage of t-BuSH-H_2SeO_3 reaction is very slow,about 40 to 150 times slower than that for n-BuSH-H_2SeO_3 reaction. In order to obtain measurable rate constants, it is necessary to use high concentrations of 2-methyl-2-propanethiol. Otherwise, the kinetic studies of this part of work were carried out using the same conditions as for the second stage of n-BuSH-H_2SeO_3 reaction. However, some of the kinetic data were obtained by monitoring the reaction at 300 nm instead of 310 nm in order to get a greater absorbance change. Table III gives the rate data for the second stage of t-BuSH-H_2SeO_3 reaction at various pH's. It is seen that the reaction rate of this stage, like the second stage in n-BuSH-H_2SeO_3 reaction is first order in mercaptan.

The pH-rate profile for the second stage of this reaction is of the same general shape as that for the second stage of n-BuSH-H_2SeO_3 reaction and is shown in Figure 7.

Final Products of Thiol-Selenious Acid Reaction

The reaction of 2-methyl-2-propanethiol with selenious acid was carried out in 60% dioxane at room temperature for two hours. The solvent was then removed under reduced pressure and the residue was washed with water. The mixture was extracted with methylene chloride and dried over magnesium sulfate. Removal of the methylene chloride under reduced

pressure gave an oily yellow crude product. The crude product was then distilled under vacuum, giving of product I. The NMR spectrum of product I in deuteriochloroform has a sharp peak at δ 1.42 which corresponds to the protons of t-butyl groups in di-t-butylselenotrisulfide. However, the spectrum also has two small singlets at δ 1.48 and 1.62. Thin layer chromatography of procuct I on silica gel using hexane as eluent shows one large spot and one tiny spot. These indicated that there must be two fractions in product I, one di-t-butylselenotrisulfide and one unknown. Product I was chromatographed on a column of silica gel using hexane as the eluent. After the first fraction was collected, the eluent was gradually changed to ether to elute the second fraction. Fraction I as separated by chromatography has a clean singlet at δ 1.42 in its NMR spectrum. Its mass spectrum shows a molecular ion at M = 258, and in line with the fact that Se isotopes of atomic weight 76, 77, 78 and 82 are also present in significant natural abundance, the peak at 258 (^{80}Se) is accompanied by less prominent peaks at M = 254, 255, 256 and 260. The mass spectrum clearly indicates that fraction I is di-t-butylselenotrisulfide. The isolated yield of this compound from this particular experiment was 0.57 mmole per mmole of selenious acid.

In a later experiment in which the crude product was chromatographed directly rather than being distilled before

chromatography, the first fraction eluted was found to consist a mixture of t-BuSSeSBu-t and t-BuSSBu-t. From the total weight of the fraction and the intensity of the NMR signal at δ 1.42 due to the selenotrisulfide, the yield of the selenotrisulfide was calculated to be 0.78 mmole per mmole of selenious acid.

The second fraction had an NMR spectrum that consisted of two singlets of equal intensity at δ 1.46 and δ 1.62. Its mass spectrum shows a molecular ion at M = 210. The infrared spectrum of this fraction has two distinct strong absorption bands at 1300 and 1100 cm^{-1} indicating the presence of an $-SO_2$ group. The proton-decoupled ^{13}C NMR spectrum of the fraction shows that there are four different types of carbon present in this compound. These give singlets at 23.74 ppm, 31.52 ppm, 56.29 ppm and 68.02 ppm. The singlets at 23.74 and 31.52 ppm are of much stronger intensity than those at 56.29 and 68.02 ppm. The elemental analysis of the compound gives the following results: C: 45.96%, H: 8.69%, S: 30.70%, which when taken together with the mass spectrum indicates a molecular formula of $C_8H_{18}S_2O_2$.

The above evidence suggest that this fraction is t-butyl 2-methyl-2-propanethiolsulfonate, t-$BuS(O)_2$SBu-t. The yield of this thiolsulfonate from the experiment just outlined was 0.11 mmole per mmole of selenious acid.

However, in a later experiment in which the crude product was chromatographed directly without a prior vacuum distillation, the amount of t-Bu$S(O)_2$SBu-t formed per mole of H_2SeO_3 reacted was considerably larger, 0.162 mmole per mmole of H_2SeO_3.

An authentic sample of this thiolsulfonate was prepared following the method given by Asakawa and coworkers[24]. However, the crude product obtained by this method was a mixture and had to be chromatogfaphed on silica gel to get pure t-butyl 2-methyl-2-propanethiolsulfonate. The compound obtained by this method has exactly the same infrared, NMR, and mass spectrum as the second fraction isolated from product I.

In another experiment the crude product was examined directly by NMR. There are four singlets seen: (1) those at δ 1.62 and 1.46 due to t-Bu$S(O)_2$SBu-t; (2) one at δ 1.42, due to t-BuSSeSBu-t; and (3) one at δ 1.32, due to t-BuSSBu-t. From the ratio $2 \times \delta_{1.62}/(\delta_{1.46+1.42} - \delta_{1.62})$ one can estimate the molar ratio [t-BuSO_2SBu-t]/[t-BuSSeSBu-t] in the product and from the ratio $\delta_{1.32}/(\delta_{1.46+1.42} - \delta_{1.62})$ one can estimate the molar ratio [t-BuSSBu-t]/[t-BuSSeSBu-t] in the isolated product mixture. These molar ratios are calculated from from the NMR data to be: [t-Bu$S(O)_2$SBu-t]/[t-BuSSeSBu-t] = 0.3 and [t-BuSSBu-t]/[t-BuSSeSBu-t] = 0.23. The mole ratio of thiolsulfonate to selenotrisulfide indicated by this experiment may be a more accurate measurement of the

relative yield of thiolsulfonate to selenotrisulfide than that from either of the chromatography isolation experiments. Because t-BuSSBu-t is considerably more volatile than either of the other reaction products there is almost certainly some loss of this product during the evaporation of solvent and methylene chloride. Thus, the yield of 0.23 mole of disulfide per mole of selenotrisulfide is undoubtedly significantly lower than the actual yield of disulfide. This was shown by another experiment in which a known amount of t-BuSSBu-t was deliberately added at the start of the reaction. It was found that only 0.60 mole of disulfide/mole added initially was recovered at the end as judged by the NMR analysis method. If one assumes that the same sort of recovery is achieved for the disulfide formed in the reaction, the actual yield of disulfide/mole of selenotrisulfide is probably about 0.4 mole/mole selenotrisulfide.

The reaction of 1-butanethiol with selenious acid shows the same general behavior as the 2-methyl-2-propanethiol-selenious acid reaction except the yield percentage of n-butyl 1-butanethiolsulfonate is much lower and the yield of disulfide is much higher. Chromatography of the crude product obtained by removing methylene chloride under reduced pressure gave fraction I, which is a mixture of n-butyldisulfide and di-n-butylselenotrisulfide, and fraction II, which is n-butyl 1-butanethiolsulfonate.

The NMR spectrum of fraction I indicated that the ratio of n-BuSSBu-n to n-BuSSeSBu-n in the fraction as isolated was 0.47:1.0. However, since disulfide is undoubtedly lost during work-up due to its volatility being greater than that of the other reaction products, the actual yield of n-BuSSBu-n per mole of selenotrisulfide is undoubtedly significantly larger than this. From the weight of fraction I and the percentage of it indicated by NMR to be selenotrisulfide, the yield of selenotrisulfide is calculated to be 0.78 mmole per mmole of selenious acid. From the weight of fraction II, which is, of course, n-BuS(O)$_2$SBu-n, the yield of thiolsulfonate was estimated to be 0.04 mmole/mmole of selenious acid.

An authentic sample of n-butyl 1-butanethiolsulfonate was also prepared by the method mentioned before. It has the same infrared and NMR spectrum as fraction II isolated from the products of the n-BuSH-H_2SeO_3 reaction.

In view of the product studies of these reactions, we wondered if perhaps some t-butyl 2-methyl-2-propanethiolsulfonate might have been formed as one of the final products in the t-BuSH-$PhSeO_2H$ reaction studied earlier by Kice and Lee[20] and had been overlooked because of its small amount in the product study they carried out on this reaction, where they reported finding only PhSeSBu-t and t-BuSSBu-t as products. It was important to find out whether or not

any of this thiolsulfonate was being formed in t-BuSH-$PhSeO_2H$ reaction because this would provide an indication as to whether or not the reaction after the second stage of the t-BuSH-H_2SeO_3 reaction proceeds by the same type mechanism as the reaction after the first stage of the t-BuSH-$PhSeO_2H$ reaction.

The final products of the t-BuSH-$PhSeO_2H$ reaction were reexamined by carrying out the reaction using exactly the same procedure as in the previous work[20]. The infrared spectrum of the crude final products did not show any indication of the strong absorption bands at 1310 and 1110 cm^{-1} associated with the SO_2 group in the thiolsulfonate. Thin layer chromatography of the final products on silica gel using hexane showed only a single spot. However, when a very small amount of authentic t-butyl 2-methyl-2-propanethiolsulfonate was added to the final products and another thin layer chromatogram run, two spots were evident. This shows that no significant amount of t-$BuS(O)_2SBu$-t is produced as a product in the reaction of t-BuSH with $PhSeO_2H$.

Studies Aimed at Isolation of an Intermediate from the End of the Second Stage of the t-BuSH-H_2SeO_3 Reaction

The ultraviolet spectrum of the reaction following the end of the second stage of the t-BuSH-H_2SeO_3 reaction in 60% dioxane has been examined. It has been found that an absorption peak shifts gradually from 262 nm to 275 nm in about

4 hours with a simultaneous increase of a broad band ranging from 285 nm to 370 nm. After that both absorbances still increase but the rate is rather slow. After 11 hours, the spectrum remains essentially unchanged. In previous work[20] on the reaction between bezeneseleninic acid and t-butyl mercaptan, the intermediate, PhSe(O)SBu-t was successfully isolated by allowing one mole of t-BuSH to react with one mole of $PhSeO_2H$ for a period of time long enough to maximize formation of the intermediate and then freezing the reaction mixture and removing the solvent and any excess mercaptan by lyophilization. Using this technique, attempts were made to try to isolate the intermediate from the end of the second stage of the reaction between t-BuSH and selenite.

Reaction mixtures of 2-methyl-2-propanethiol (0.8 mmole) and selenious acid (0.3 mmole) were allowed to react in 60% dioxane for 8 hours and 16 hours, respectively, before freezing the solution and carrying out the lyophilization. The lyophilization flasks were kept at -25°C by a dry ice-acetone bath throughout the entire lyophilization, in order to prevent any thermal decomposition of the intermediate. The residues remaining at the end of lyophilization were stored at -80°C until use. However, even with these precautions, the attempts to isolate an intermediate failed. The two residues gave the same NMR spectrum in deutero-

acetone at -20°C as the crude final product from the reaction of t-BuSH and selenious acid. The NMR spectrum consists of a singlet at δ 1.41 and two small peaks at δ 1.43 and 1.59 respectively. Upon the solution being warmed from -20°C to +20°C and kept at that temperature for one hour, the spectrum underwent no change. This meant that the residues were rather stable and did not undergo other reactions.

Another experiment of trying to stop the reaction of t-BuSH and selenious acid at the end of the first stage of the reaction was run by allowing the reaction mixture to react for 3 minutes, then following exactly the same freezing and lyophilizing procedure. The residue in this case had the same NMR spectrum as the previous two, which suggests that during the stage of lyophilization the first intermediate must either undergo further reaction or else revert back to thiol and selenious.

DISCUSSION

Sulfhydryl compounds react readily with selenite. A number of years ago Painter[15] suggested three possible courses for this reaction:

$$2\ RSH + H_2SeO_3 \longrightarrow RS\text{-}\overset{\overset{O}{\|}}{Se}\text{-}SR + 2\ H_2O \qquad (8)$$

$$4\ RSH + H_2SeO_3 \longrightarrow RS\text{-}Se\text{-}SR + RSSR + 3\ H_3O \qquad (2)$$

$$4\ RSH + H_2SeO_3 \longrightarrow 2\ RSSR + Se + 3\ H_2O \qquad (9)$$

In 1968, Ganther[14] found that the combining ratio for thiols and selenious acid was 4:1 by spectrophotometric analysis. Two selenotrisulfides, selenodicysteine and selenodimercaptoethanol, were also isolated successfully and showed the chromophore of -S-Se-S- group in the ultraviolet absorption spectra. These facts conformed to the reaction of Eq. 2 proposed by Painter, and the usual stoichiometry of the reaction is now thought to be as shown in Eq. 2.

In the present work the kinetics of the reaction of both 1-butanethiol (n-BuSH) and 2-methyl-2-propanethiol (t-BuSH) with selenious acid have been examined in 60% dioxane over the pH range 0.2-8.1. The formation of the selenotrisulfide is kinetically biphasic, and under most conditions the rate

of the first stage is much faster than the rate of the second. The first stage of the reaction is first order in the stoichiometric concentrations of both thiol and the selenious acid. The intermediate (I) produced in the first stage of the reaction is then consumed in a reaction that is first order in itself and first order in thiol. This second stage of the reaction cannot directly produce the selenotrisulfide and so presumably it must lead to the formation of a second intermediate (II) which then goes on to react with additional thiol and eventually lead to the observed final products in reactions that are not amenable to kinetic investigation, presumably because consumption of II is rapid compared to its rate of formation. Thus, without concern yet for the ionization state of the various reactants in the different steps, the kinetic situation for the RSH-H_2SeO_3 reaction can be represented as:

$$H_2SeO_3 \xrightarrow[k_a]{RSH} InH(I) \xrightarrow[k_b]{RSH} II \xrightarrow{2RSH} RSSeSR + \text{other products} \qquad (10)$$

Relative Magnitudes of k_a and k_b under Various Conditions

For the reaction of t-BuSH with selenious acid $k_a >> k_b$ through the pH range 0.2-8.1, but for n-BuSH, although $k_a >> k_b$ at pH = 0.2-7.0, above pH = 7.8 $k_b > k_a$.

Several aspects of the behavior of the first stage of t-BuSH-H_2SeO_3 reaction show that this reaction is actually

an equilibrium, i.e.

$$\underline{t}\text{-BuSH} + H_2SeO_3 \underset{k'_{-o}}{\overset{k'_o}{\rightleftharpoons}} I \qquad (11)$$

and not an irreversible process. If the reaction went to completion, then $A_\infty - A_o$, the total absorbance change for the first stage of the reaction, should be independent of $[\underline{t}\text{-BuSH}]_o$ as long as the thiol is present in stoichiometric excess over selenite. However one actually finds that $A_\infty - A_o$ for the first stage, whether measured at 262 nm or 253 nm, increases with increasing concentration of $\underline{t}$-BuSH up to about $[\underline{t}\text{-BuSH}]_o = 0.04$ M.

The kinetic behavior of the first stage also indicates it is an equilibrium. If the reaction were irreversible the experimental first-order rate constant, k_a, would follow the relationship $k_a = k_o[RSH]$. However, if the reaction is an equilibrium, then k_a will be equal to the sum of the rates of the forward and the reverse reactions, i.e. $k_a = k'_{-o} + k'_o[RSH]$. The kinetic behavior of the first stage of the reaction of $\underline{t}$-BuSH with selenite is indeed found to follow this relationship:

$$k_a = k'_{-o} + k'_o[\underline{t}\text{-BuSH}] \qquad (12)$$

These results indicate that the first stage of the

t-BuSH-H_2SeO_3 reaction is an equilibrium:

$$\text{t-BuSH} + H_2SeO_3 \underset{k'_{-o}}{\overset{k'_o}{\rightleftharpoons}} \text{InH} \qquad (13)$$

From the values of k'_o and k'_{-o} it is possible to calculate the equilibrium constant $K_{eq} = k'_o/k'_{-o}$ for Eq. 13. This is found to have a value of about 600 M^{-1} in media of pH = 1-4.5 and a value about half as large for solution of pH 5.5. It seems likely that the first stage of the n-BuSH-H_2SeO_3 reaction is also reversible. However, in this case the equilibrium constant is enough larger than that for the t-BuSH-H_2SeO_3 reaction that $k'_o[RSH] >> k'_{-o}$ for the conditions used for the kinetic runs, i.e. n-BuSH = 0.001-0.003 M.

pH-Rate Profiles for the Forward Reaction of the First Stage

The pH-rate profiles of the n-BuSH-H_2SeO_3 reaction and the t-BuSH-H_2SeO_3 reaction, Fig. 5 and Fig. 6, have the same general shape. The rate increases with decreasing pH from 7 down to 5. Below pH = 5 the rate levels off at a constant value. The pK_{a_1} of selenious acid in 60% dioxane can be estimated to be 5.0. The pH-rate profiles in Figs. 5 & 6 are what one would expect if the only reaction of kinetic importance throughout the entire pH range is that of RSH with undissociated H_2SeO_3. The concentration of selenite present as $[H_2SeO_3]$ varies with pH in the following manner:

$$[H_2SeO_3] = \left(\frac{a_{H^+}}{K_a + a_{H^+}} \right) C_{\text{selenite}} \qquad (14)$$

where $C_{selenite}$ is the total concentration of selenite present in the solution, K_a is the dissociation constant for the ionization, $H_2SeO_3 \rightleftharpoons HSeO_3^- + H^+$, and a_{H^+} is the hydrogen ion activity of the solution.

When $a_{H^+} >> K_a$, $[H_2SeO_3] = C_{selenite}$; but once $a_{H^+} < K_a$ $[H_2SeO_3] = C_{selenite}(a_{H^+}/K_a)$, and the amount of selenite present as H_2SeO_3 will decrease linearly with decreasing a_{H^+}.

The fact that the rate of the first stage of the thiol-selenite reactions decreases linearly with pH above pH = 5 means that the reaction of RSH with $HSeO_3^-$ is never kinetically important compared to reaction of RSH with H_2SeO_3 at any pH within the pH range studied. This is true even though at the upper end of the pH range studied $[H_2SeO_3]/[HSeO_3^-]$ has become as small as 10^{-3}.

If the forward reaction of the first stage of the reaction between a thiol and selenious acid has a general mechanism of the type shown in Scheme IV:

$$\begin{array}{l} H_2SeO_3 + RSH \xrightarrow{k_o} InH \\ \quad\uparrow\downarrow K_a \qquad\qquad\qquad (I) \\ HSeO_3^- + H^+ \end{array}$$

Scheme IV

one would expect to find the following dependence of rate on

pH for k_a:

$$k_a = k_o \frac{a_{H^+}}{K_a + a_{H^+}} \qquad (15)$$

where K_a is the acid dissociation constant for H_2SeO_3 and is assumed to be 1×10^{-5} ($pK_a = 5$) in 60% dioxane. Calculated pH-rate profiles using this equation are shown in Figs. 5 & 6, using the following k_o values:

	k_o
for n-BuSH	420
for t-BuSH	52

One sees that the experimental data for the rates of the forward reaction of the first stage are in both cases fitted extremely well by the curves calculated from Eq. 15 using the values of k_o and K_a indicated. The mechanism shown in Scheme IV does therefore correctly account for the observed pH-rate profiles for k_a.

pH-Rate Profile for the Second Stage of the Thiol-Selenite Reaction

The U-shaped pH-rate profiles of the second stage of n-BuSH-H_2SeO_3 and t-BuSH-H_2SeO_3 reactions, (Fig. 4 and Fig. 7) remind one of the pH-rate profile that is observed for the first stage of the reaction between thiols and $PhSeO_2H$. The mechanism for the first stage of the RSH-$PhSeO_2H$ reaction has been shown to be as outlined in Scheme V[20].

$$PhSeO_2H + H^+ \underset{}{\overset{K_1}{\rightleftharpoons}} PhSeO_2H_2^+ \qquad (16a)$$

$$PhSeO_2H \overset{K_a}{\rightleftharpoons} PhSeO_2^- + H^+ \qquad (16b)$$

$$RSH + PhSeO_2H_2^+ \xrightarrow{k_H} \underset{OH\ \ H}{\overset{OH}{PhSe-\overset{+}{S}-R}} \overset{fast}{\underset{-H^+}{\rightleftharpoons}} \qquad (17a)$$

$$RSH + PhSeO_2H \xrightarrow{k_o} \underset{OH}{\overset{OH}{Ph-Se-SR}} \xrightarrow{-H_2O} PhSeSR \qquad (17b)$$

$$RS^- + PhSeO_2H \xrightarrow{k_{RS^-}} \underset{O-}{\overset{OH}{PhSe-S-R}} \overset{+H^+}{\underset{fast}{\rightleftharpoons}} \qquad (17c)$$

$$RSH \overset{K_{RSH}}{\rightleftharpoons} RS^- + H^+ \qquad (18)$$

Scheme V

At low pH the dominant reaction kinetically is reaction of RSH with the protonated form of the seleninic acid, $PhSeO_2H_2^+$, and the rate of the reaction increases as a_{H^+} increases. At intermediate pH's the most important reaction is reaction of RSH with undissociated $PhSeO_2H$. At higher pH's the reaction of RS^- with $PhSeO_2H$ becomes the most important reaction. The mechanism in Scheme V leads to the following predicted dependence of rate on pH:

$$k_1 = \frac{k_H K_1 a_{H^+}^2 + k_o a_{H^+} + k_{RS^-} K_{RSH}}{K_a + a_{H^+}} \qquad (19)$$

An equation for k_1 of the form shown in Eq. 19 leads to the type of U-shaped pH-rate profile that is found for the reaction.

The similarity in the shape of the pH-rate profiles for the second stage of the thiol-selenite reaction with that for the first stage of the thiol-$PhSeO_2H$ reaction suggests that a mechanism for the second stage of the thiol-selenite reaction having the general form shown in Scheme VI will explain the particular type of pH-rate profile observed.

$$InH + H^+ \underset{}{\overset{K_1'}{\rightleftharpoons}} InH_2^+ \qquad (20a)$$

$$InH \overset{K_a'}{\rightleftharpoons} In^- + H^+ \qquad (20b)$$

$$RSH + InH_2^+ \xrightarrow{k_H'} \underset{-H^+}{\overset{\text{fast}}{\longrightarrow}} - \qquad (21a)$$

$$RSH + InH \xrightarrow{k_o'} II \qquad (21b)$$

$$RS^- + InH \xrightarrow{k_{RS}^-'} \underset{\text{fast}}{\overset{+H^+}{\longrightarrow}} \qquad (21c)$$

$$RSH \overset{K_{RSH}}{\rightleftharpoons} RS^- + H^+ \qquad (22)$$

Scheme VI

This mechanistic scheme will, of course, give the following dependence of rate on pH for k_b:

$$k_b = \frac{k_H' K_1' a_{H^+}^2 + k_o' a_{H^+} + k_{RS^-}' K_{RSH}}{K_a' + a_{H^+}} \qquad (23)$$

Different pathways for formation of intermediate II dominate the kinetics in different pH region. Below pH = 2, it is reaction of the undissociated thiol with the protonated intermediate I, InH_2^+. At these pH's $a_{H^+} >> K'_a$, and so in this pH region $k_b = k'_H k'_1 a_{H^+}$, and the rate increases linearly with increasing a_{H^+}, as is observed. By pH = 2, the rate of reaction of RSH with InH_2^+ has become slow enough, due to the decrease in $[InH_2^+]$ with a decrease in a_{H^+}, that reaction of RSH with InH becomes the dominant reaction. This reaction, whose rate is independent of pH in this pH region, is the dominant process only over a narrow pH range (~2-4), because above pH = 4 the fraction of RSH present as RS^-, although still very small, becomes large enough so that reaction of RS^- with InH, k'_{RS^-}, becomes the important route kinetically to II. In the pH region where the k'_{RS^-} term is the major contributor to k_b, k_b will be given by $k_b = k'_{RS^-} K_{RSH}/(K'_a + a_{H^+})$. This means that in this region k_b will increase with increasing pH (decreasing a_{H^+}) up to the point where $K'_a > a_{H^+}$. At that point, k_b will become independent of pH with a value equal to $k'_{RS^-} K_{RSH}/K'_a$.

In the mechanism in Scheme VI, k'_H, k'_o and k'_{RS^-} should of course be different depending on whether the thiol is n-BuSH or t-BuSH. At the same time, it is likely that K'_a for the ionization of InH to In^- and H^+ should have effectively the same value for R = n-Bu as for R = t-Bu.

The curves in Figs. 4 and 7 have been calculated from Eq. 23 using a value of $K'_a = 5 \times 10^{-6}$ for each case and values of the other constants as shown in the table below.

	$k'_H K'_1$	k'_o	$k'_{RS} - K_{RSH}$
for n-BuSH	6	0.06	2.7×10^{-6}
for t-BuSH	0.15	0.0012	2.0×10^{-8}

As one can see, the calculated curves from Eq.23 fit the actual experimental data very well in each case. Obviously a mechanism of the general type shown in Scheme VI is indicated for the second stage of the thiol-selenite reaction.

One most impost conclusion from this curve fitting is that intermediate I, formed in the first stage from RSH and H_2SeO_3, must be a species that posseses a readily ionizable to proton, i.e. to fit the kinetics this species must be able to ionize as $InH \rightleftharpoons In^- + H^+$. Furthermore, the pK_a of InH has to be 5.3 in order to get a proper fit of the calculated curves from Eq. 23 to the experimental results.

Since the pK_a of H_2SeO_3 in 60% dioxane is 5.0, this suggests that InH must be a species of selenium acid that might reasonably be expected to have a pK_a very similar to that for HOSe(O)OH. We also know, of course, that it is formed in the first stage from one molecule of thiol and

one of H_2SeO_3. For these reasons the structure for intermediate I would seem almost certainly to be RS-SeO_2H.

A species with the structure RS-SeO_2H could be expected to have a pK_a quite close to that for $HOSeO_2H$. Since RS is inductively somewhat less electron withdrawing than OH, it might be expected to be a somewhat weaker acid, and that is also what is found.

When one compares the rate constant for the reaction of n-BuSH with H_2SeO_3 with that for the reaction of the undissociated thiol with $PhSeO_2H$, it is found that the reaction rate of the former is much greater than that of the latter by a factor of more than 1000. One also finds that the rate of reaction of the undissociated thiol with RS-SeO_2H in the second stage of the n-BuSH-H_2SeO_3 reaction is much slower than the rate of reaction of n-BuSH with H_2SeO_3. This is shown below:

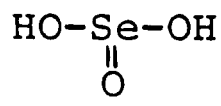
$$\text{n-BuSH} + \text{HO-SeO}_2\text{H} \xrightarrow{k_o' = 400\ M^{-1}s^{-1}} \text{n-BuS-SeO}_2\text{H} + H_2O \qquad (24)$$

$$\text{n-BuSH} + \text{Ph-SeO}_2\text{H} \xrightarrow{k_o = 0.25\ M^{-1}s^{-1}} \text{n-BuS-Se(=O)Ph} + H_2O \qquad (25)$$

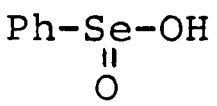
$$\text{n-BuSH} + \text{n-BuS-SeO}_2\text{H} \xrightarrow{k_o'' = 0.06\ M^{-1}s^{-1}} \text{n-BuS-Se(=O)SBu-n} + H_2O \qquad (26)$$

The striking rate differences between the above reactions

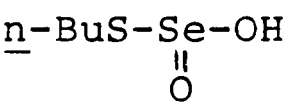
leads one to ask this question: Why does selenious acid (III) react so much faster than either benzeneseleninic acid or n-$BuSSeO_2H$ with 1-butanethiol?



III



IV



V

The only difference between these three reactant species is that a hydroxyl group is attached to the selenium atom in species III instead of the phenyl group in species IV or the n-BuS group in species V. Why should the presence of a hydroxyl group be able to accelerate the rate so much? Certainly it can not be due to its inductive effect since n-BuS-SeO_2H doesn't react at a much different rate than $PhSeO_2H$.

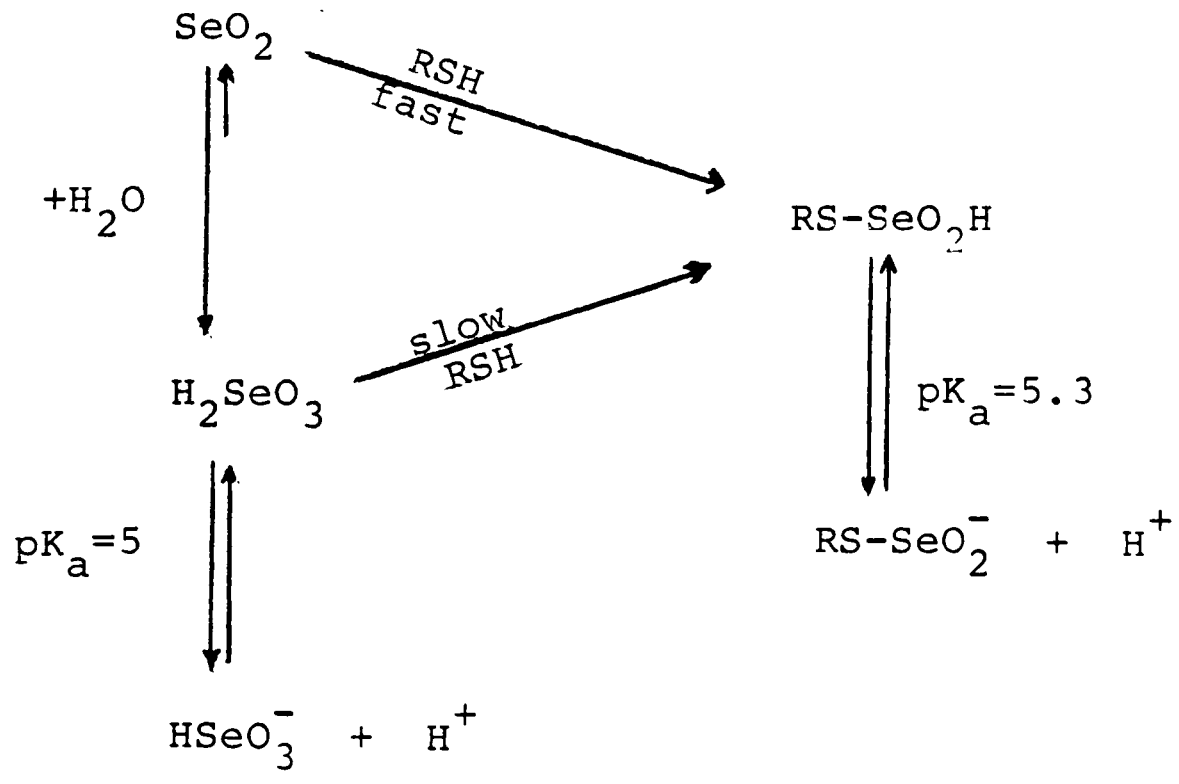
The suggestion is that $HOSeO_2H$ reacts much faster than IV or V because it can do something the other species can't, namely, lose water to give a small amount of its dehydration product, selenium dioxide, in equilibrium with it in the aqueous dioxane solution. If SeO_2 is much more reactive toward the thiol than $HOSeO_2H$ itself then one can see how selenious acid can be much more reactive than IV or V toward n-BuSH.

It has been shown[25,26] that sulfurous acid, H_2SO_3, exists in such a form that most of it is $SO_2 + H_2O$, i.e. they

are in equilibrium with each other following Eq. 27:

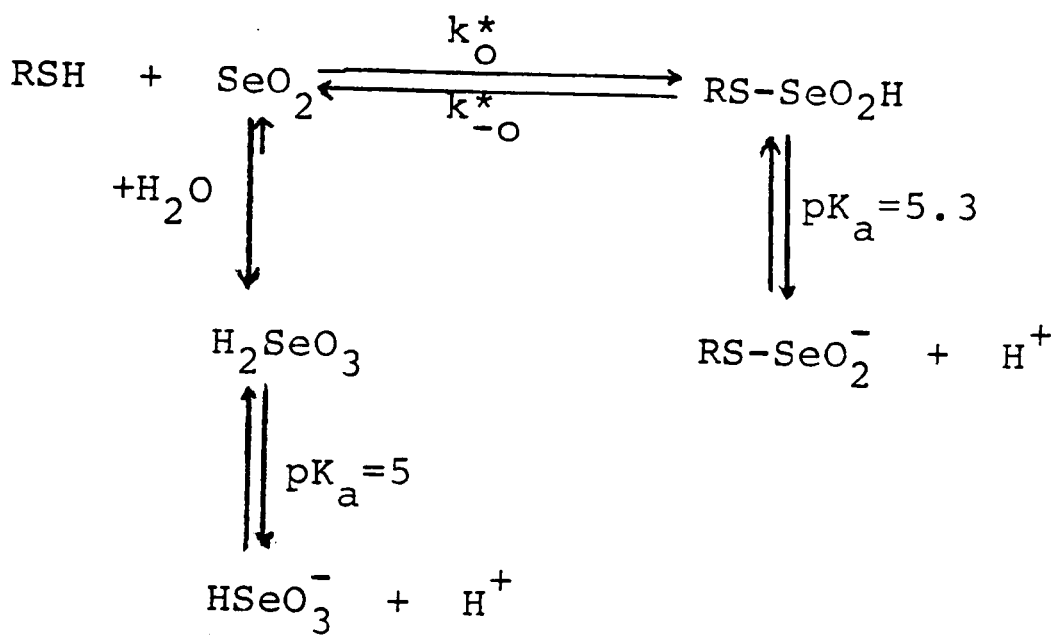
$$H_2SO_3 \rightleftharpoons SO_2 + H_2O \qquad (27)$$

This fact suggests that even though H_2SeO_3 has been shown[27] to be mostly in the hydrated form. there could still be a few percent of SeO_2 at equilibrium. Thus it seems reasonable to suggest that the reactive entity is not H_2SeO_3 but the small amount of SeO_2 in equilibrium with it. Addition of the thiol to SeO_2 is apparently very rapid. This addition generates a product that has an ionizable proton with pK_a=5.3 in 60% dioxane. This is almost the same as the pK_a of H_2SeO_3, which is consistent with the structure RS-SeO_2H for this product. The following scheme shows reactions of selenium dioxane and selenious acid with thiol and relationship between them.



Scheme VII

As we have already seen, the addition of the thiol is reversible. Therefore, the actual complete mechanism for the first stage of the thiol-selenite reaction is presumably as shown in Scheme VIII.



(28)

Scheme VIII

The rate ratio between n-BuSH and t-BuSH for the first stage of RSH-H_2SeO_3 reaction, $k^*_{o,n\text{-BuSH}}/k^*_{o,t\text{-BuSH}}$, has a value about 8 throughout the entire pH range studied. When one compares this ratio with that of the first stage of the RSH-$PhSeO_2H$ reaction, where $k_{n\text{-BuSH}}/k_{t\text{-BuSH}} \simeq 5$, one finds that they are almost the same. This fact indicates both reactions involve the same type of reaction, i.e. the nucleophilic attack by sulfur on selenium. except that the rate of the thiol-selenite reaction is accelerated by the presence of a small amount of SeO_2 in equilibrium with H_2SeO_3.

Although Scheme VI explains the particular shape of the pH-rate profile for the second stage of the thiol-selenite reaction quite satisfactorily, it does not tell us exactly how the thiol attacks $RSSeO_2H_2^+$ or $RSSeO_2H$, or how RS^- attacks $RSSeO_2H$. For the purposes of discussion let us consider for the moment only the reaction between $RSSeO_2H$ and the undissociated thiol, RSH. There would seem to be three possible ways in which RSH could attack $RSSeO_2H$. All of these lead to intermediates that can go on to yield the correct final products, so that one can't very well distinguish which is correct on this basis. The three possible modes of attack of RSH on $RSSeO_2H$ are as follows:

(A) attack on sulfur:

$$RSH + RS\text{-}SeO_2H \longrightarrow RSSR + HO\text{-}SeOH \qquad (29)$$

(B) attack on selenium:

$$RSH + RS\text{-}SeO_2H \longrightarrow RS\text{-}\underset{\underset{O}{\|}}{Se}\text{-}SR + H_2O \qquad (30)$$

(C) attack on oxygen:

$$RSH + RS\text{-}SeO_2H \longrightarrow RSOH + RS\text{-}SeOH \qquad (31)$$

Reactions involving nucleophilic attack on the sulfur of a t-BuS group are known to be much slower than the rate of attack of the same nucleophile on an analogous n-BuS group. Rate differences of $k_{n\text{-BuS}}/k_{t\text{-BuS}}$ of up to 10^6 have been ob-

served[31]. The fact that $k_{\underline{n}\text{-BuS}}/k_{\underline{t}\text{-BuS}} = 40$ for the reaction of RSH with $RSSeO_2H$ seems to be too small to be consistent with the mechanism (A) where RSH attacks $RSSeO_2H$ on sulfur, and would appear to rule out Eq. 29 as the mechanism. That leaves us to decide between attack on selenium (Eq. 30) and on oxygen (Eq. 31) as the probable mechanism for the reaction of RSH with $RSSeO_2H$.

The pH-rate profile for the second stage of the thiol-H_2SeO_3 reaction has similar shape to the one observed in the first stage of RSH-$PhSeO_2H$ reaction, except that the plateau of the former is somewhat lower than that of the latter. As mentioned earlier, this type of pH-rate profile indicates there are three different reactions that each dominate in different pH regions. By assuming the dissociation constant of RSH, K_{RSH}, has a value of 10^{-14} and K_a's of RS-SeO_2H and Ph-SeO_2H are 10^{-5} and 6×10^{-8} in 60% dioxane, one can estimate k_{RS}-'s, the reaction rates between thiolate ion with RS-SeO_2H and Ph-SeO_2H. Since the magnitude of protonation constants of both RS-SeO_2H and $PhSeO_2H$ are not known, we cannot make any reliable estimate of k_H, the rate constant of reaction between thiol and the protonated acid. A comparison of all rates of RSH-$RSSeO_2H$ reaction and RSH-$PhSeO_2H$ reaction in these three different pH regions shows that they are in relative the same order. The estimated rate constants are summarized in the following table:

Reaction	K_1k_H	k_o	k_{RS^-}
$PhSeO_2H$-n-BuSH	10	0.25	9.2×10^7
n-$BuSSeO_2H$-n-BuSH	6	0.06	9.6×10^7
$PhSeO_2H$-t-BuSH	2.8	0.06	5.4×10^7
t-$BuSSeO_2H$-t-BuSH	0.15	0.0013	2.0×10^6

This suggests that the reaction of thiol and RS-SeO_2H might have the same mechanism as the reaction of thiol and Ph-SeO_2H, i.e. attack of sulfur on selenium as found by Kice and Lee[20] in studies of that reaction.

Furthermore the rate ratio of n-BuSH and t-BuSH in RSH-$RSSeO_2H$ reaction, $k_{n\text{-BuS}}/k_{t\text{-BuS}}$, ranging from 40-100, is somewhat greater than that of the RSH-$PhSeO_2H$ reaction, which has a value of 5. This is because in the reaction of thiol and $PhSeO_2H$, the group neighboring the selenium is always the same, i.e. a phenyl group, while in the reaction of thiol and RS-SeO_2H, the neighbor is n-BuS when the attacking thiol is n-BuSH and t-BuS when the attacking thiol is t-BuSH. Since a t-BuSH group should be more sterically hindering to attack on the selenium than the small n-BuSH group, one can understand why attack of RSH on the selenium of RS-SeO_2H should show a somewhat larger $k_{n\text{-BuS}}/k_{t\text{-BuS}}$ rate ratio than for the attack of the two thiols on the selenium of $PhSeO_2H$.

On the other hand, if attack of RSH on $RSSeO_2H$ were on oxygen there is no obvious reason why $k_{\underline{n}\text{-BuS}}/k_{\underline{t}\text{-BuS}}$ shuold be larger than for attack of RSH on PhSeO H since the oxygen is far enough away from the RS group that the steric size of R should have little effect on rate. Thus, it appears that the second step of the thiol-H_2SeO_3 reaction involves attack of the thiol on the selenium atom of $RSSeO_2H$ to give RSSe(O)SR.

The question we are left with at this point is how RSSe(O)SR reacts with thiol to get the final products, RSSeSR and RSSR. There are two possible ways that one can get the same products, they are shown below:

(A) attack on sulfur:

$$\begin{array}{l} RS\text{-}Se\text{-}SR \\ \quad\| \\ \quad O \end{array} + RSH \longrightarrow RSSR + RS\text{-}SeOH \qquad (32)$$

$$RS\text{-}SeOH + RSH \longrightarrow RSSeSR + H_2O \qquad (33)$$

(B) attack on oxygen:

$$\begin{array}{l} RS\text{-}Se\text{-}SR \\ \quad\| \\ \quad O \end{array} + RSH \longrightarrow RSSeSR + RSOH \qquad (34)$$

$$RSOH + RSH \longrightarrow RSSR + H_2O \qquad (35)$$

It has been found[19] that RSSeH is not a stable species and will undergo decomposition to give thiol and metallic sele-

nium. If reaction goes in the following way:

$$RS\text{-}SeOH + RSH \longrightarrow RSSeH + RSOH \qquad (36)$$

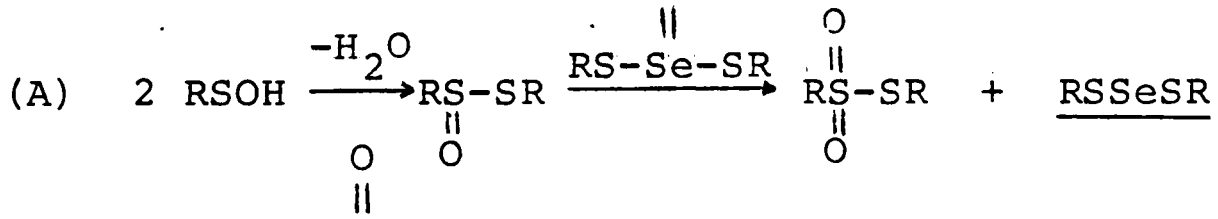
one would expect to get some metallic selenium in the final products. However, this is not the case, the only selenium-containing product is RSSeSR in the RSH-H_2SeO_3 reaction.

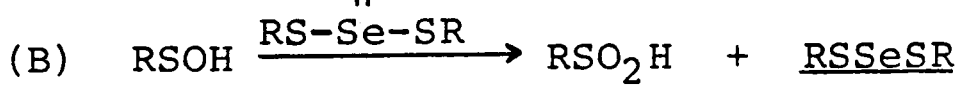
In the reaction of t-BuSH and selenious acid, less t-BuSSBu-t was found than predicted. Instead, quite a large amount of t-butyl 2-methyl-2-propanethiolsulfonate, t-BuS(O)$_2$SBu-t, was found in the final products. It seems that in order to form t-BuS(O)$_2$SBu-t, one must start with t-BuSOH, i.e. attack of sulfur on oxygen seems to be more likely. However in the t-BuSH-$PhSeO_2H$ reaction where we know that PhSe(O)SBu-t is formed as an intermediate, we could show that no t-BuS(O)$_2$SBu-t is formed as a product. This indicates that the attack of thiol on oxygen in t-BuSSe(O)SBu-t is apparently much easier relative to the attack on sulfur than in PhSe(O)SBu-t. This also explains why the intermediate II, t-BuSSe(O)SBu-t, could not be isolated at the end of the second stage by lyophilization.

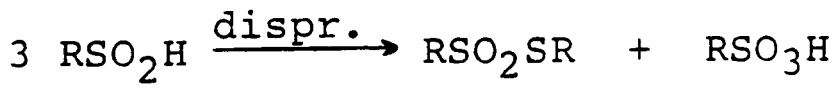
In the n-BuSH-H_2SeO_3 reaction, the situation is different; the yield of n-BuS(O)$_2$SBu-n is much lower than the yield of t-BuS(O)$_2$SBu-t in the t-BuSH-H_2SeO_3 reaction. Two explanations seem possible: (1) n-BuSH also attacks on oxygen to form n-BuSOH, but most n-BuSOH reacts with n-BuSH to give

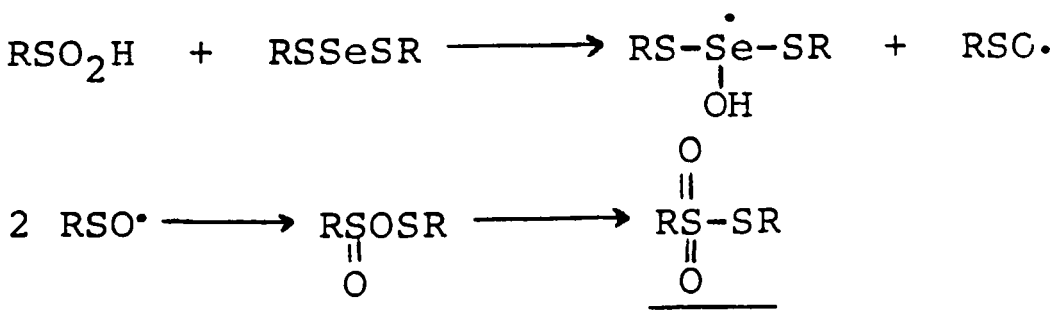
n-BuSSBu-n. Only a small amount of it goes to the product n-BuS(O)$_2$SBu-n because n-BuSOH, which is not as hindered as t-BuSOH, is more likely to react with another molecule of n-BuSH to form the disulfide. (2) Attack occurs mainly on sulfur, and formation of n-BuS(O)$_2$SBu-n is due to a side reaction. Unfortunately, there is no way to tell which of these alternatives is more likely until further work is done.

Although we know from what has been mentioned above that t-BuSOH once formed goes on to form t-BuS(O)$_2$SBu-t, we don't know at this point exactly how this happens, because there are several possible ways that one can get to the same product. Three reasonable speculations are shown below:









(C) $RSOH + RS-\underset{\underset{O}{\|}}{Se}-SR \longrightarrow RSO\cdot + RS-\underset{\underset{OH}{|}}{\dot{S}e}-SR$ (44)

$RS-\underset{\underset{OH}{|}}{\dot{S}e}-SR \longrightarrow RS-SeOH + RS\cdot$ (45)

$RS\cdot + RS-\underset{\underset{O}{\|}}{Se}-SR \longrightarrow RS-\underset{\underset{OSR}{|}}{\dot{S}e}-SR \longrightarrow RSSeSR + RSO\cdot$ (46)

$RSO\cdot$ (from eq. 44) + $RSO\cdot$ (from eq. 46) $\longrightarrow RSO_2SR$ (47)

$RS-SeOH + RSH \longrightarrow RSSeSR + H_2O$ (48)

Mechanism (A) seems the least likely possibility since it has been observed[28,29], that the dehydration of t-BuSOH to give t-BuS(O)SBu-t is rather slow. It seems unlikely that t-BuS(O)SBu-t would be formed rapidly enough for there to be sufficient t-BuS(O)SBu-t present while there was still enough t-BuSSe(O)SBu-t left in the solution. This leaves us to decide whether mechanism (B) or (C) occurs under the reaction conditions. However, in order to determine which mechanism is more likely to happen, one needs to do some more studies. These will require enough time that they can not be done as part of the present work and must be left for a future study.

Thus, from the kinetic and product studies conducted in the present work, one can depict the mechanism of the $RSH-H_2SeO_3$ reaction up to the end of the second stage with reasonable certainty. The first stage involves a reversible

reaction of selenium dioxide with thiol to form an intermediate RS-SeO_2H. The second stage of the reaction involves the attack of thiol on the selenium of the intermediate, $RSSeO_2H$, formed in the previous stage, to give the second intermediate, RSSe(O)SR.

Although further reactions of this intermediate with n-BuSH and t-BuSH proceed to give different product mixtures, one can almost definitely say that the further reaction in the t-BuSH-H_2SeO_3 reaction involves the attack of t-BuSH on the oxygen of the intermediate. Not only the presence of t-$BuS(O)_2SBu$-t in the final products of the t-BuSH-H_2SeO_3 reaction, but also the intermediate studies by lyophilization, showed consistency with this proposed mechanism.

However, the mechanism of the formation of t-$BuS(O)_2$-SBu-t from t-BuSOH is still unknown and thus provides a new area of organoselenium chemistry to be explored.

EXPERIMENTAL SECTION

Purification of Materials

1,4-Dioxane was purified by the following procedure. A mixture of 40 ml of concentrated hydrochloric acid, 3 liters of dioxane and 300 ml of water was refluxed under nitrogen for 12 hours. Potassium hydroxide pellets were then added to the cooled solution until no more would dissolve and a separate layer formed. The upper dioxane layer was decanted and dried with fresh potassium hydroxide. The dried dioxane was then refluxed over sodium overnight until the surface of the metal remained bright. The pure dioxane was fractionally distilled (b.p. 102°C) from sodium and then frozen and stored at -20°C to prevent the formation of peroxides prior to use.

All water used in kinetic studies was doubly distilled. Both thiols (Aldrich), 1-butanethiol and 2-methyl-2-propanethiol, used in kinetic runs were fractionally distilled prior to use.

Procedure for Kinetic Runs Using Conventional Spectrophotometry

A stock solution of selenious acid was prepared by dissolving a carefully weighed amount of selenium dioxide (Alfa) in a known volume of water. A stock solution of the appropriate thiol was prepared immediately prior to use by dis-

solving a weighed amount of the thiol in a known volume of pure dioxane.

A stock solution of 3.5 ml of 60% dioxane containing the proper amount of either perchloric acid or the appropriate buffer was placed in a 1-cm spectrophotometer cell and thermostated at 25°C in the cell compartment of a Cary Model 17 spectrophotometer for 20 minutes. The desired amount of selenious acid was then added by a microsyringe. The reaction was then initiated by adding via another microsyringe the proper amount of the stock solution of the thiol. The increase in absorbance at a certain wavelength was followed. Pseudo first-order rate constants for each run was determined from the slope of plots of $\log(A_\infty - A_t)$ vs. time.

In the case of the second stage of either reaction of n-BuSH or t-BuSH with selenious acid, the change of optical density was monitored at 310 nm. Since the rate of this stage is slow when compared with the first stage of the reaction, it can be treated kinetically as an independent reaction. For most of the runs involving the first stage of the thiol-H_2SeO_3 reaction, the absorbance change versus time was followed at 262 nm. However, in the n-BuSH case at pH > 7, the rate of the first stage becomes comparable to the rate of the second stage and for these runs the absorbance change versus time was followed at 248 nm, a wavelength where the conversion of the intermediate from the first stage to the

final products shows an isobestic point.

Procedure for Kinetic Runs Using Stopped-Flow Spectrophotometry

All solutions were freshly prepared immediately prior to use. The procedure was as follows. A stock solution of thiol (n-BuSH or t-BuSH) with a concentration of 0.004 M (or higher) in 60% dioxane containing the appropriate buffer was prepared and placed in on of the reservoir syringes of a Durrum-Gibson Model D-110 stopped flow spectrophotometer. A stock solution of selenious acid (1.05×10^{-4} M) in 60% dioxane was prepared and placed in the other reservoir syringe. To start the reaction, the solutions were mixed using the stopped flow device and the course of the reaction was monitored at a certain wavelength on the storage oscilloscope. For the case of n-BuSH-H_2SeO_3 reaction, the reaction was monitored at either 262 nm or 248 nm. Both gave consistent results. In the case of t-BuSH, the increase in absorbance with time was followed at 253 nm or 273 nm.

Product Studies of the t-BuSH-H_2SeO_3 Reaction

To 1.11 g (10 mmoles) of selenium dioxide in 50 ml of 60% dioxane was added dropwise with constant stirring 4.51 g (50 mmoles) of t-BuSH. The reaction mixture was allowed to stand for 2 hours at room temperature, and the solvent (dioxane) was removed under reduced pressure. The solution

was washed twice with 100 ml of water. This solution was then extracted with two 50 ml portions of methylene chloride. The methylene chloride extracts were dried over anhydrous magnesium sulfate. The magnesium sulfate was filtered off, and the solvent was removed under reduced pressure. The residue was distilled *in vacuo* (b.p. 59°C/0.3 mm) to give 1.7 g of product I.

Product I was chromatographed on a column of silica gel using hexane, hexane-ether (3:1, 1:1, 1:2) and ether as eluents. Elution with hexane gave 1.47 g of *t*-butylselenotrisulfide, IR: 2960, 1460, 1365, 1160 cm^{-1}. NMR ($CDCl_3$): δ 1.42(s). MS: m/e 258 (M^+ for ^{80}Se). The presence of molecular ion peaks for the other selenium isotopes (^{76}Se, ^{77}Se, ^{78}Se, and ^{82}Se) at 254, 255, 256 and 260 provided a characteristic fingerprint showing the presence of an Se atom in the molecule.

Elution with ether gave 0.23 g (fraction II). The various spectral properties of the compound were as follows: IR: 2960, 1460, 1395. 1365, 1300, 1155, 1100,1020 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.46(s), 1.62(s). ^{13}C NMR: δ 23.47(s), 31.52(s), 56.29(s), 68.02(s). MS: m/e 210 (M^+).

On the basis of the spectral data fraction II was assigned the formula *t*-Bu$S(O)_2$S*t*-Bu. The correctness of this assignment was confirmed by elemental analysis.

Anal. calcd. for $C_8H_{18}O_2S_2$: C, 45.70; H, 8.63; S, 30.45. Found: C, 45.96; H, 8.69; S, 30.70.

When the crude products were chromatographed directly on silica gel without an initial vacuum distillation, a somewhat larger yield of both t-BuSSeSBu-t and t-BuS(O)$_2$SBu-t was obtained. The initial fraction (Fraction I) could also be shown to contain some t-BuSSBu-t as indicated by the presence of a singlet at δ 1.32. Much of the disulfide formed in the reaction was apparently lost during work-up due to its greater volatility. From the weight of fraction I and the relative intensity of the δ 1.42 singlet in the NMR, the yield of selenotrisulfide from this experiment was found to be 0.78 mmole/mmole of selenious acid reacted. The yield of responded to t-BuSSBu-t. This was proven by the occurrence of an intensified peak when an authentic sample of t-BuSSBu-t was added to the t-BuSH-H_2SeO_3

The NMR spectrum of the crude products obtained immediately after removal of the methylene chloride under reduced pressure showed a singlet at δ 1.32 besides singlets at δ 1.42, 1.46 and 1.62. This singlet was less intense relative to the singlet at δ 1.42 (0.23:1) and corresponded to t-BuSSBu-t. This was proven by the occurrence of an intensified peak when an authentic sample of t-BuSSBu-t was added to the t-BuSH-H_2SeO_3 reaction.

Product Studies of the n-BuSH-H_2SeO_3 Reaction

1-Butanethiol (4.51 g, 50 mmoles) was added dropwise to a solution of 50 ml of 60% dioxane containing 1.11 g (10 mmoles) of selenium dioxide. The reaction mixture was allowed to stand for two hours at room temperature. The same purification procedures used in the t-BuSH-H_2SeO_3 reaction were then followed except that the vacuum distillation was omitted. After removing the methylene chloride under reduced pressure there was 2.92 g of residue. Chromatography of this residue on silica gel gave 2.68 g of fraction I and 0.08 g of fraction II. The NMR spectrum of fraction I consisted of a triplet at δ 0.82~1.06, a multiplet at δ 1.22~1.88, a triplet at δ 2.62~2.80 and a triplet at δ 2.90~3.05. Comparison of this spectrum with the NMR spectrum of an authentic sample of n-BuSSBu-n showed that the triplet at δ 2.62~2.80 is due to n-BuSSBu-n, and that the triplet at δ 2.90~3.05 is therefore due to n-BuSSeSBu-n. From the ratio of the intensities of the two triplets, the ratio of n-BuSSBu-n/n-BuSSeSBu-n in fraction I can be determined. The ratio of n-BuSSBu-n to n-BuSSeSBu-n found in this fraction was 0.47 : 1.

Spectral properties of fraction II were as follows:
IR: 2960, 1460, 1415, 1390, 1330, 1230, 1130, 1100, 910 cm^{-1}.
NMR ($CDCl_3$): δ 0.84~1.1 (overlapped triplets, 6H); δ 1.26~2.08 (multiplet, 8H); δ 3.06~3.42 (overlapped triplets, 4H).

This information, combined with the results found in the product studies of the t-BuSH-H_2SeO_3 reaction made it easy to assign to this compound the structure n-$BuS(O)_2SBu$-n. This was confirmed by a comparison of the spectral properties of fraction II with those of an authentic sample of n-$BuS(O)_2SBu$-n, the preparation of which is described in a subsequent section of the Experimental Section.

Preparation of t-Butyl 2-Methyl-2-propanethiolsulfonate

To 10.0 g (56.2 mmoles) t-butyldisulfide (Aldrich) dissolved in 17 ml of acetic acid and kept at 0°C was added 7 ml of 30% hydrogen peroxide. After 12 hours, this mixture was extracted twice with 20 ml portions of chloroform. The extracts were then washed with saturated sodium bicarbonate solution and dried over magnesium sulfate. After the magnesium sulfate was filtered off, the solvent was removed under reduced pressure. The residue (7.67 g) had an NMR spectrum that consisted of two singlets of equal intensity at δ 1.39 and 1.57. Its infrared spectrum was as follows: 2960, 1460, 1395, 1370, 1220, 1165, 1020 cm^{-1}. Block[30] has proven that this compound has the structure t-BuS(O)SBu-t.

t-Butyl 2-methyl-2-propanethiolsulfinate (7.67 g) obtained from the above oxidation was then dissolved in 16 ml of acetic acid and 4.5 ml of 30% hydrogen peroxide was added. The reaction mixture was allowed to stand overnight

with stirring. The solution was then extracted with 20 ml portions of chloroform. The extracts were washed with saturated sodium bicarbonate solution and dried over magnesium sulfate. The magnesium sulfate was filtered off and the solvent was removed under reduced pressure. Chromatography of 1.74 g of the residue on silica gel using benzene as eluent gave 0.73 g fraction I and 0.38 g fraction II. Fraction I showed the following spectral properties: IR: 2960, 1460, 1395,1365, 1300, 1155, 1100, 1020 cm^{-1}; NMR ($CDCl_3$): δ 1.42(s), 1.5(s); MS: m/e 242 (M^+); and was assigned to the structure t-$BuS(O)_2SBu$-t.

Fraction II had the same infrared spectrum as t-$BuS(O)_2$-SBu-t. However, its NMR and mass spectra were different: NMR ($CDCl_3$): δ 1.46(s), 1.62(s); MS: m/e 210 (M^+). These spectral properties are exactly the same as those of the material considered to have the structure of t-$BuS(O)_2SBu$-t that is formed as one of the products of the t-$BuSH$-H_2SeO_3 reaction.

Preparation of n-Butyl 1-Butanethiolsulfonate

To 10.0 g (56.2 mmoles) n-butyldisulfide (Aldrich) dissolved in 17 ml of acetic acid was added 7 ml of 30% hydrogen peroxide. The mixture was kept at 40°C with constant stirring for 12 hours. The product was oxidized by additional 30% hydrogen peroxide and the same procedures used in the preparation of t-$BuS(O)_2SBu$-t were then followed except for the chromatography.

The residue obtained from the very last stage after removal of the solvent had the same spectral properties: IR: 2960, 1460, 1415, 1390, 1330, 1230, 1130, 1100, 910 cm^{-1}; NMR ($CDCl_3$): δ 0.84~1.1 (overlapped triplets, 6H); δ 1.26~2.08 (multiplet, 8H); δ 3.06~3.42 (overlapped triplets, 4H); as those of the material considered to have the structure of n-$BuS(O)_2SBu$-n that is formed as a minor product in the n-$BuSH$-H_2SeO_3 reaction.

Attempted Isolation of the Intermediate from the End of the Second Stage of the t-$BuSH$-H_2SeO_3 Reaction

2-Methyl-2-propanethiol (0.0722 g, 0.8 mmole) was dissolved in pure dioxane to make a solution of total volume of 2 ml. This solution was then added dropwise with stirring to a solution of 0.0333 g (0.3 mmole) of selenium dioxide dissolved in 8 ml of 60% dioxane. Based on the observation that the ultraviolet spectrum of the reaction mixture was essentially unchanged after 11 hours, the solution was allowed to stand for 16 hours at room temperature. The reaction mixture was then quickly frozen at -78°C using dry ice-acetone. The solvent was then removed by lyophilization. To prevent any possible thermal decomposition of of the intermediate, the lyophilization flask was cooled externally at -25°C with an ice bath throughout the latter stages of the lyophilization. The light yellow residue remaining at the end of the lyophilization was kept at

-80°C until use. The residue has a low-temperature NMR spectrum (in acetone-d_6) consisting a singlet at δ 1.41 and two small peaks at δ 1.43 and 1.59 respectively. When the solution was warmed up to 20°C and kept at this temperature for one hour, the spectrum was essentially the same.

Experiments in which the reaction mixture was allowed to stand for a shorter periods of time (either 8 hours, or only 3 minutes) prior to being frozen and lyophilized were also carried out. The residues at the end of these experiments had the same NMR spectrum and showed the same behavior after being warmed up to 20°C.

TABLE I

Kinetics of the Second Stage of the Reaction of 1-Butanethiol with Selenious Acid in 60% Dioxane at 25°C

Reaction Conditions	pH	$10^4[H_2SeO_3]_o$, M	$10^3[RSH]$, M	$10^3 \times k_{ii}$, S^{-1}	$k_b = k_{ii}/[RSH]$ $M^{-1}S^{-1}$
0.6 M $HClO_4$	0.22	2.1	2.0	10	5.0
			3.9	21	5.4
			5.9	35.5	5.8
0.3 M $HClO_4$	0.52	2.1	3.9	6.8	1.74
			5.9	10.5	1.78
			7.7	13.6	1.76
0.1 M $HClO_4$	1.00	2.1	3.9	1.98	0.5
			5.9	2.89	0.49
0.03 M $HClO_4$	1.52	2.1	3.9	0.64	0.16
			7.7	1.14	0.15

TABLE I. (Continued)

Reaction Conditions	pH	$10^4[H_2SeO_3]_o$, M	$10^3[RSH]$, M	$10^3 \times k_{ii}$, S^{-1}	$k_b = k_{ii}/[RSH]$ $M^{-1}S^{-1}$
0.01 M $HClO_4$	2.00	2.1	4.0	0.334	0.083
			6.0	0.453	0.075
1:1 CF_3COOH: $CF_3COO^-Na^+$ Buffer	2.8	2.1	4.0	0.34	0.085
			6.0	0.495	0.083
0.5:1 CF_3COOH: $CF_3COO^-Na^+$ Buffer	3.1	2.1	4.0	0.336	0.084
			6.0	0.502	0.084
0.25:1 CF_3COOH: $CF_3COO^-Na^+$ Buffer	3.4	2.1	4.0	0.236	0.059
			6.0	0.36	0.060
			8.0	0.456	0.057
0.38:1 $CHCl_2COOH$: $CHCl_2COO^-Na^+$ Buffer	4.5	2.1	4.0	0.43	0.11
			6.0	0.64	0.11
3.8:1 $CH_2ClCOOH$: $CH_2ClCOO^-Na^+$ Buffer	4.9	2.1	4.0	0.8	0.2
			6.0	1.19	0.2

TABLE I. (Continued)

Reaction Conditions	pH	$10^4[H_2SeO_3]_o$, M	$10^3[RSH]$, M	$10^3 \times k_{ii}$, S^{-1}	$k_b = k_{ii}/[RSH]$ $M^{-1}S^{-1}$
1.1:1 $CH_2ClCOOH$: $CH_2ClCOO^-Na^+$ Buffer	5.4	2.1	4.0	1.61	0.4
			6.0	2.38	0.4
1.3:1 $HCOOH$:$HCOO^-Na^+$ Buffer	5.9	2.0	1.99	0.874	0.44
			3.95	1.97	0.50
			5.9	2.76	0.47
0.3:1 $HCOOH$:$HCOO^-Na^+$ Buffer	6.5	2.2	4.15	2.33	0.56
			6.18	3.43	0.55
			8.2	4.4	0.54
1:1 CH_3COOH: $CH_3COO^-Na^+$ Buffer	7.4	2.1	3.9	2.0	0.51
			5.8	2.98	0.51
			7.6	3.84	0.51
0.2:1 CH_3COOH: $CH_3COO^-Na^+$ Buffer	8.1	2.1	4.0	0.825	0.21
			6.0	1.16	0.19
			8.0	1.6	0.20

TABLE II

Kinetics of the First Stage of the Reaction of 2-Methyl-2-propanethiol with Selenious Acid in 60% Dioxane at 25°C

Reaction Conditions	pH	$10^4[H_2SeO_3]_o$, M	$10^3[RSH]$, M	$10^2 \times k_i$[a], s^{-1}	$10^2 \times k_o$, s^{-1}	k_a $M^{-1}s^{-1}$
0.1 M $HClO_4$	1.00	0.53	2.0	19		
			4.0	30.8		
			6.0	40.8		
			8.0	51.4	9	53
1:1 CF_3COOH: $CF_3COO^-Na^+$ Buffer	2.8	0.53	2.0	19.3		
			4.0	28.9		
			6.0	38.5		
			8.0	46.5	10.5	45.7
3.8:1 $CH_2ClCOOH$: $CH_2ClCOO^-Na^+$ Buffer	4.9	0.53	2.0	12.6		
			4.0	17.8		
			6.0	23.7		
			8.0	30.1	5.6	30.2

TABLE II. (Continued)

Reaction Conditions	pH	$10^4[H_2SeO_3]_o$, M	$10^3[RSH]$, M	$10^2 \times k_i^a$, S^{-1}	$10^2 \times k_o$, S^{-1}	k_a $M^{-1}S^{-1}$
1.1:1 $CH_2ClCOOH$: $CH_2ClCOO^-Na^+$ Buffer	5.4	0.53	2.0	5.6		
			4.0	7.37		
			6.0	9.1		
			8.0	11.6	3.3	9.8
1.3:1 HCOOH: $HCOO^-Na^+$ Buffer	5.9	1.05	2.0	1.94		
			4.0	2.63		
			6.0	3.24		
			8.0	3.94	1.3	3.3
0.3:1 HCOOH: $HCOO^-Na^+$ Buffer	6.5	1.05	1.0	0.358		
			2.0	0.447		
			4.0	0.592	0.26	0.887

TABLE II. (Continued)

Reaction Conditions	pH	$10^4[H_2SeO_3]_o$, M	$10^3[RSH]$, M	$10^2 \times k_i$[a], s^{-1}	$10^2 \times k_o$, s^{-1}	k_a $M^{-1}s^{-1}$
1:1 CH_3COOH: $CH_3COO^-Na^+$ Buffer	7.4	1.05	1.0	0.091		
			2.0	0.105		
			4.0	0.158		
			6.0	0.190		
			8.0	0.254	0.05	0.29

[a] $k_i = k_o + k_a[RSH]$

TABLE III

Kinetics of the Second Stage of the Reaction of 2-Methyl-2-propanethiol with Selenious Acid in 60% Dioxane at 25°C

Reaction Conditions	pH	$10^4[H_2SeO_3]_o$, M	10^2[RSH], M	$10^4 \times k_{ii}$, S^{-1}	$k_b = k_{ii}/[RSH]$ $M^{-1}S^{-1}$
0.6 M $HClO_4$	0.22	2.1	2.0	33.3	0.167
			3.0	48.5	0.16
			4.0	65.4	0.16
0.3 M $HClO_4$	0.52	2.1	2.0	10.5	0.053
			3.0	16.3	0.054
			4.0	22.4	0.056
0.1 M $HClO_4$	1.00	2.1	2.0	3.16	0.0158
			3.0	4.5	0.015
			4.0	5.68	0.0142
0.03 M $HClO_4$	1.52	2.1	4.0	2.03	0.0051
			6.0	2.89	0.0048

TABLE III. (Continued)

Reaction Conditions	pH	$10^4[H_2SeO_3]_o$, M	10^2[RSH], M	$10^4 \times k_{ii}$, s^{-1}	$k_b = k_{ii}/[RSH]$ $M^{-1}s^{-1}$
0.01 M $HClO_4$	2.00	2.1	4.0	0.576	0.0014
			6.0	0.825	0.0014
			8.0	1.12	0.0014
0.001 M $HClO_4$	3.00	2.1	5.0	0.656	0.0013
			6.0	0.825	0.0014
0.5:1 CF_3COOH: $CF_3COO^-Na^+$ Buffer	3.1	2.1	5.0	0.716	0.0014
			6.0	0.849	0.0014
3.8:1 $CH_2ClCOOH$: $CH_2ClCOO^-Na^+$ Buffer	4.9	2.1	4.0	0.64	0.0016
			6.0	0.93	0.00155
1.1:1 $CH_2ClCOOH$: $CH_2ClCOO^-Na^+$ Buffer	5.4	2.1	4.0	1.2	0.003
			6.0	1.78	0.003

TABLE III. (Continued)

Reaction Conditions	pH	$10^4[H_2SeO_3]_o$, M	10^2[RSH], M	$10^4 \times k_{ii}$, S^{-1}	$k_b = k_{ii}/[RSH]$ $M^{-1}S^{-1}$
1.3:1 $HCOOH:HCOO^-Na^+$ Buffer	5.9	2.1	2.0	0.738	0.0037
			3.0	1.03	0.0035
			4.0	1.33	0.0034
0.3:1 $HCOOH:HCOO^-Na^+$ Buffer	6.5	2.1	2.0	0.695	0.0035
			3.0	1.083	0.0036
			4.0	1.367	0.0034

Captions for Figures

Figure 1. Ultraviolet absorbance spectra for reaction of 1-butanethiol (2.3×10^{-3} M) with selenious acid (1.0×10^{-4} M) in 60% dioxane. (a). Scanned immediately after initiating the reaction. (b). Scanned 40 minutes after initiation.

Figure 2. Ultraviolet absorbance spectra for reaction of 1-butanethiol (2.3×10^{-3} M) with selenious acid (1.0×10^{-4} M) in 0.13 M $HClO_4$ in 60% dioxane. (a). Scanned immediately after initiating the reaction. (b). Scanned 10 minutes after initiation.

Figure 3. Ultraviolet absorbance spectra for reaction of 1-butanethiol (2.3×10^{-3} M) with selenious acid (1.0×10^{-4} M) in formate buffer (pH = 6.5) in 60% dioxane. (a). Scanned immediately after initiating the reaction. (b). Scanned 10 minutes after initiation. (c). Scanned 20 minutes of initiation. (d). Scanned 30 minutes after initiation. (e). Scanned 40 minutes after inition. (f). Scanned 70 minutes after initiation.

Figure 4. pH-rate profile of the second stage of the reaction of n-BuSH with selenious acid at 25°C in

60% dioxane: ●, rate constants, k_b, for second stage of reaction (solid curve calculated from eq. 23 using K'_a for InH = 5×10^{-6}, $k'_H K'_1 = 6$, $k'_o = 0.06$, and $k'_{RS}-K_{RSH} = 2.7 \times 10^{-6}$).

Figure 5. pH-Rate profile for the first stage of the reaction of n-BuSH with selenious acid at 25°C in 60% dioxane: ●, rate constants, k_a, for first stage of reaction (solid curve calculated from eq. 15 using K_a for $H_2SeO_3 = 1 \times 10^{-5}$ and $k_o = 420$).

Figure 6. pH-Rate profile for the first stage of the reaction of t-BuSH with selenious acid at 25°C in 60% dioxane: ●, rate constants, k_a, for first stage of reaction (solid curve calculated from eq. 15 using K_a for $H_2SeO_3 = 1 \times 10^{-5}$ and $k_o = 52$).

Figure 7. pH-Rate profile for the second stage of the reaction of t-BuSH with selenious acid at 25°C in 60% dioxane: ●, rate constants, k_b, for second stage of reaction (solid curve calculated from eq. 23 using K'_a for InH = 5×10^{-6}, $k'_H K'_1 = 0.15$, $k'_o = 0.0012$, and $k'_{RS}-K_{RSH} = 2.0 \times 10^{-8}$).

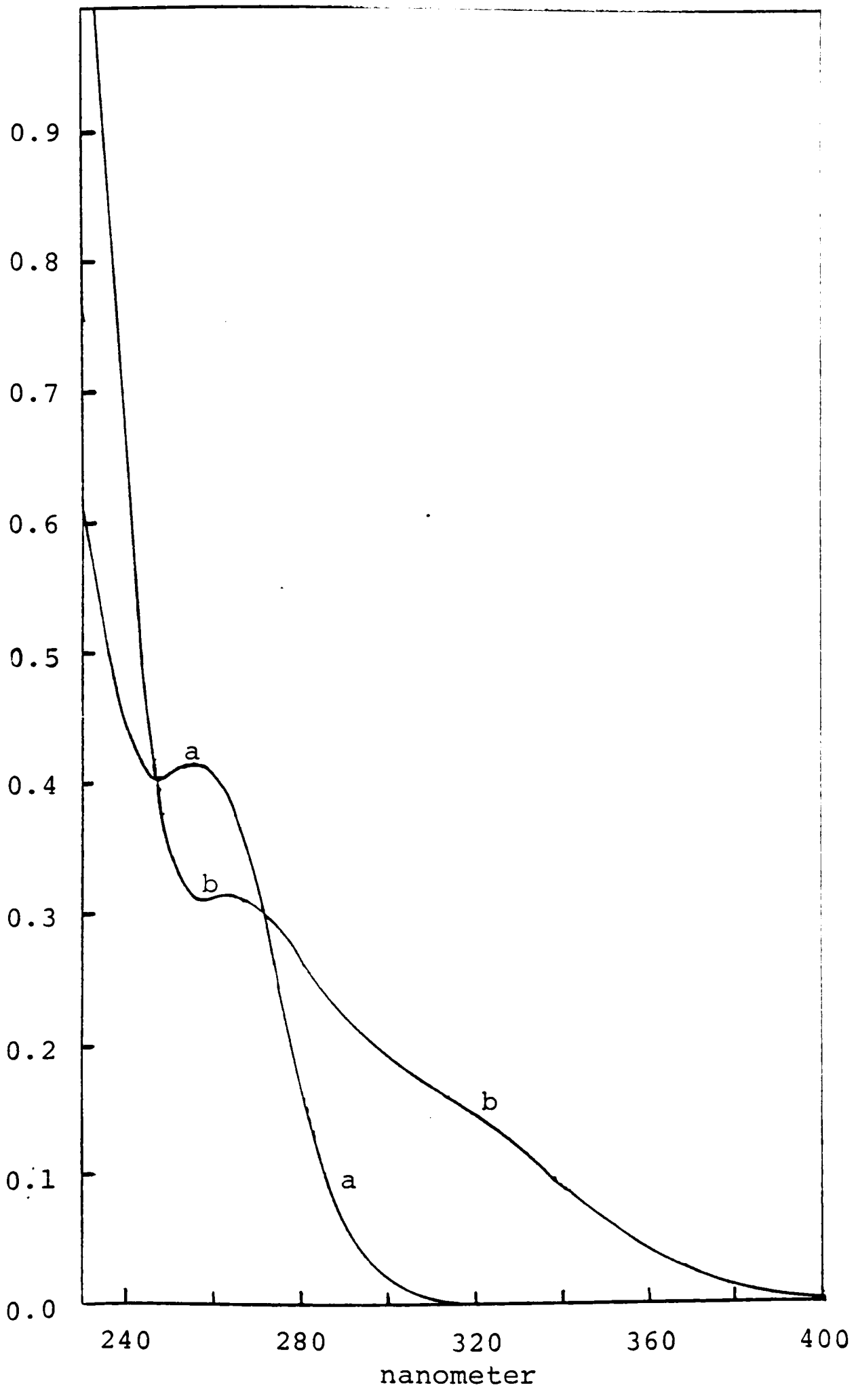


Figure 1

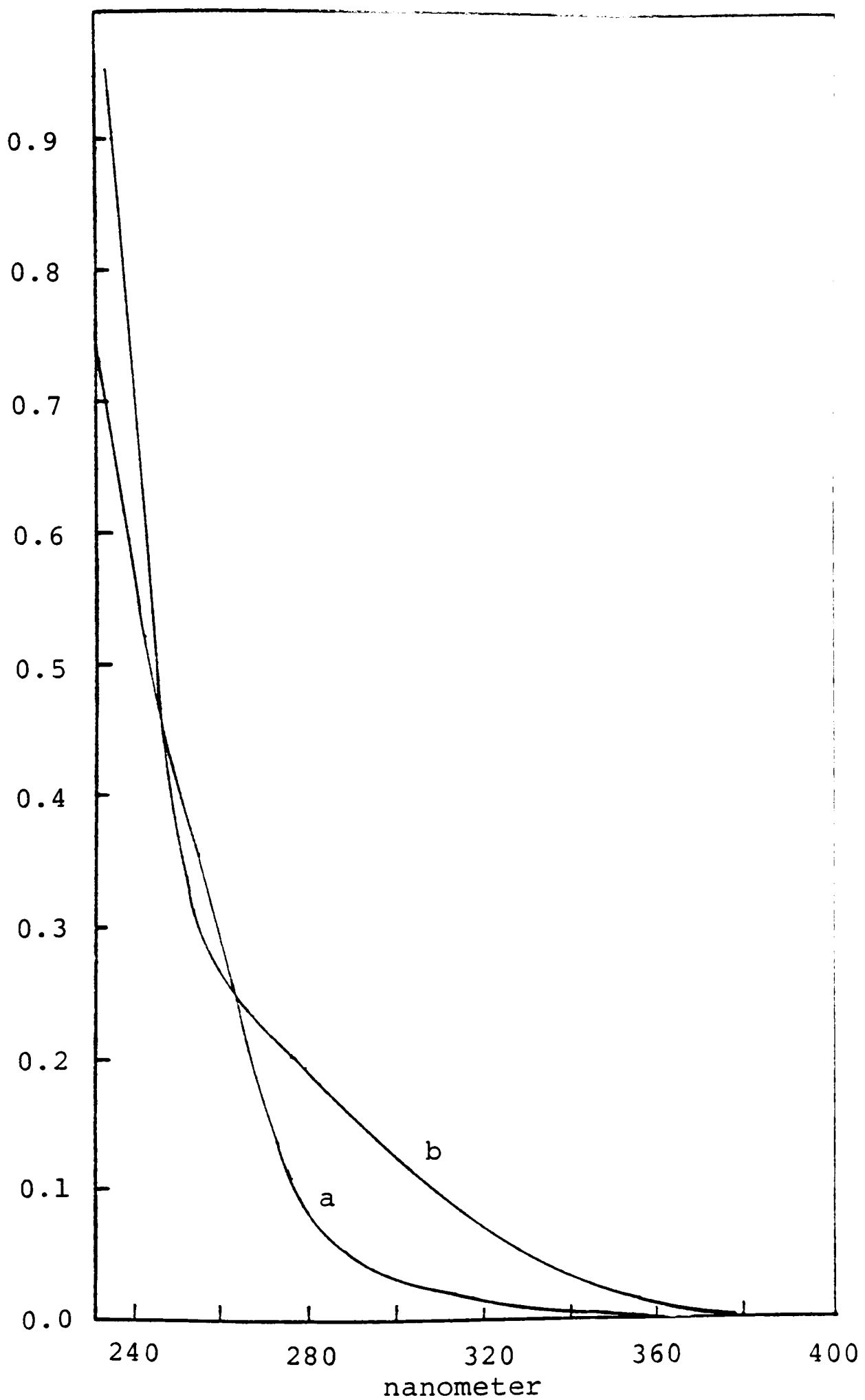
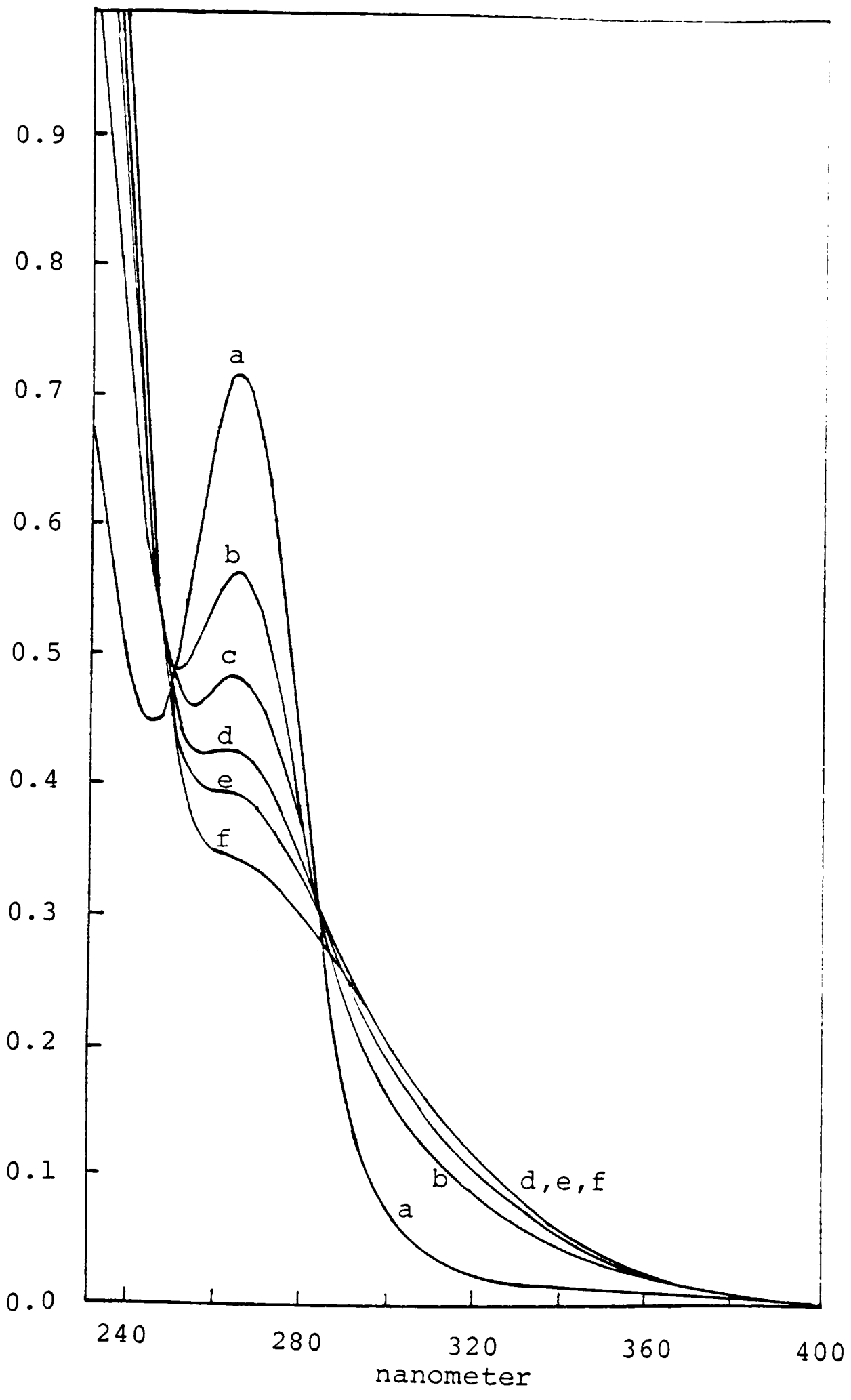


Figure 2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0.7
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a
240
280
320
360
400
nanometer

Figure 3

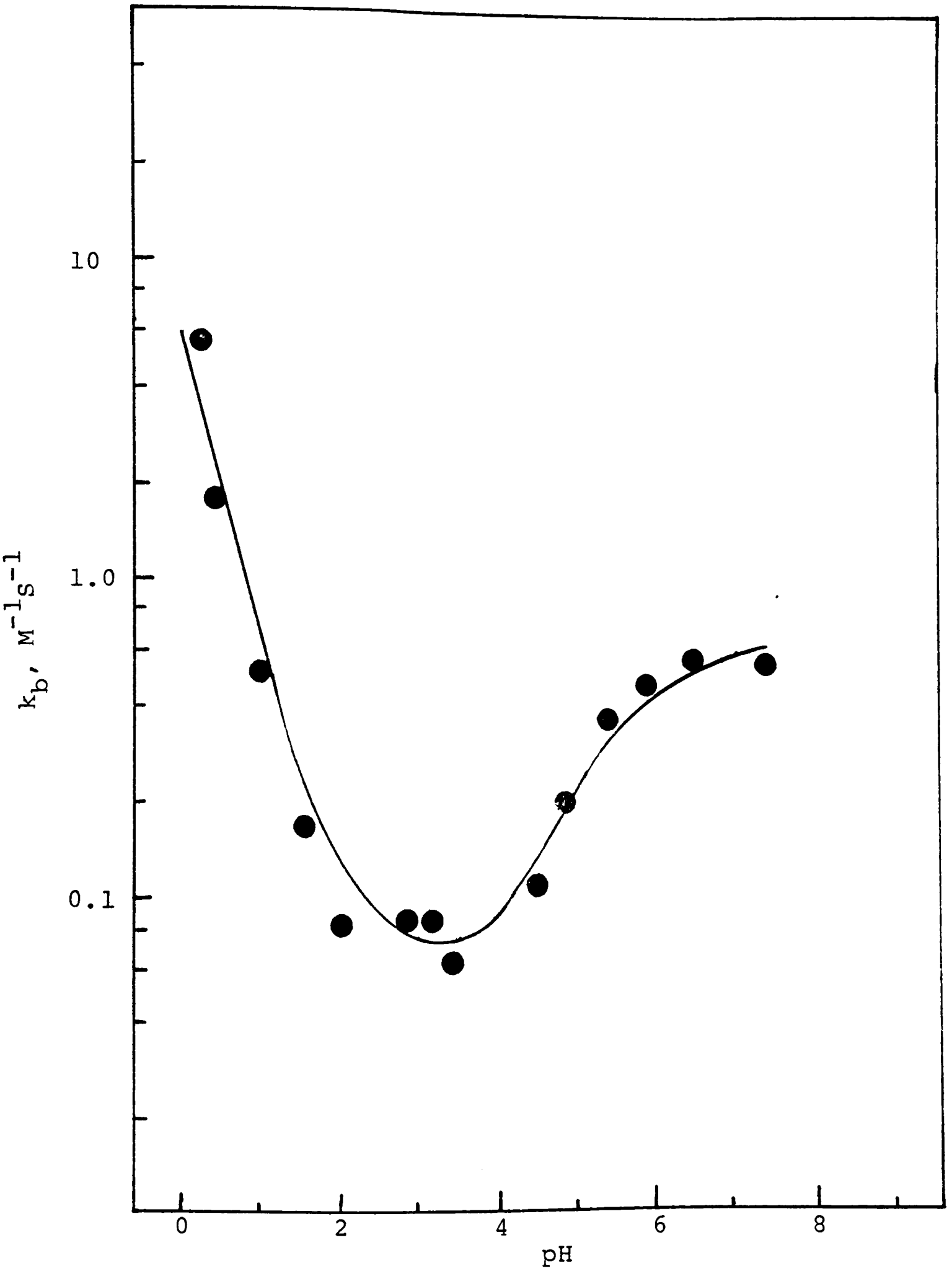

Figure 4

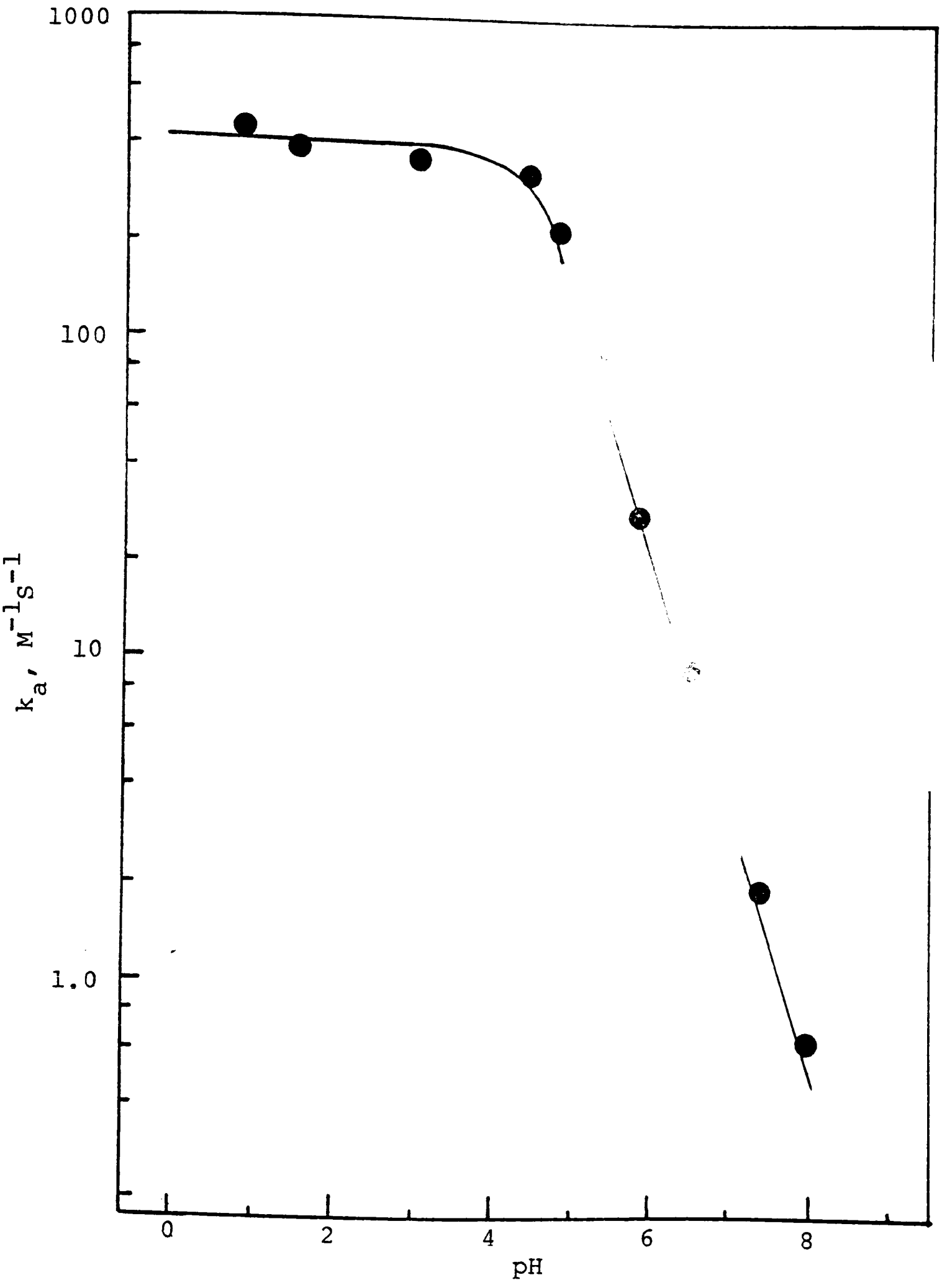

Figure 5

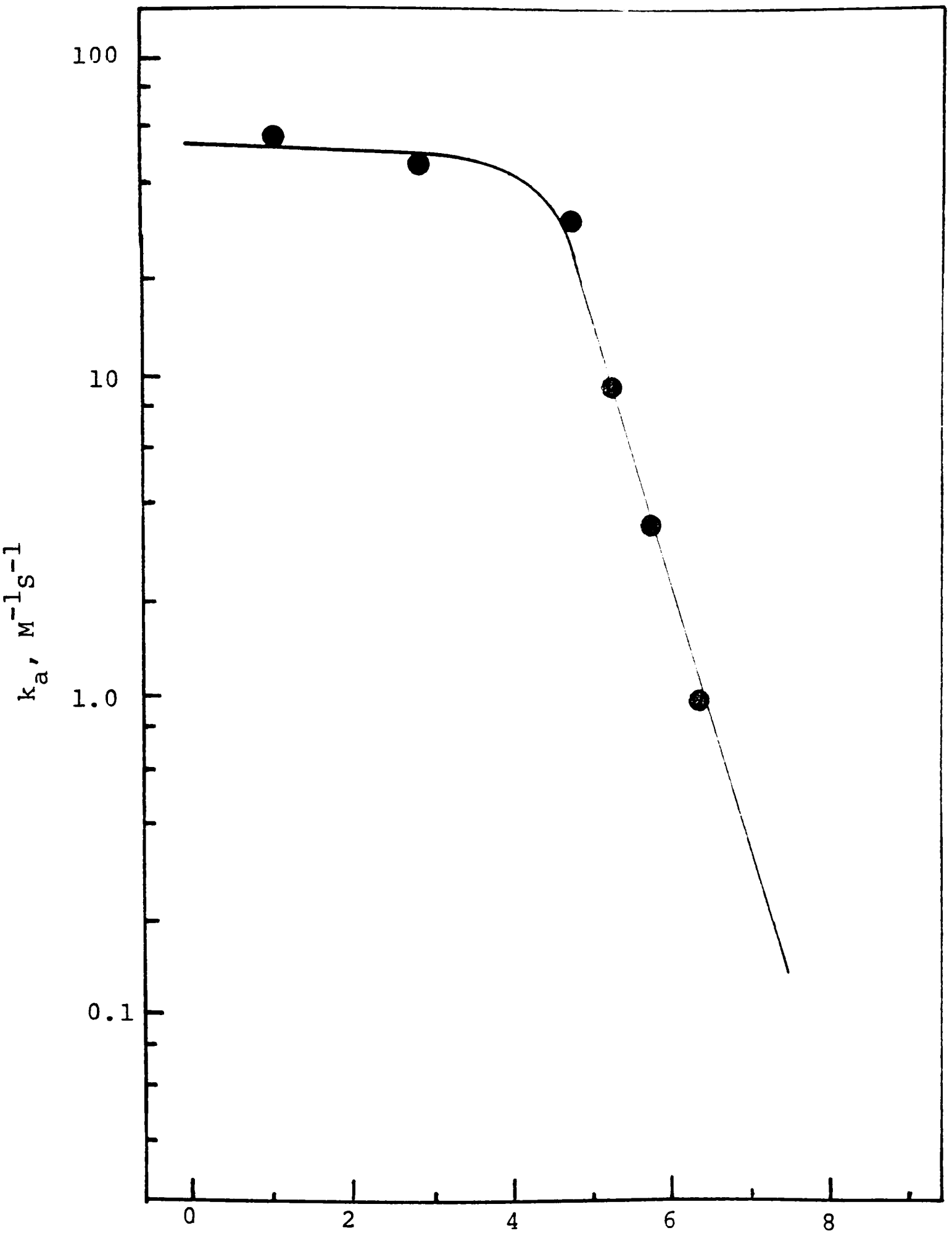

Figure 6

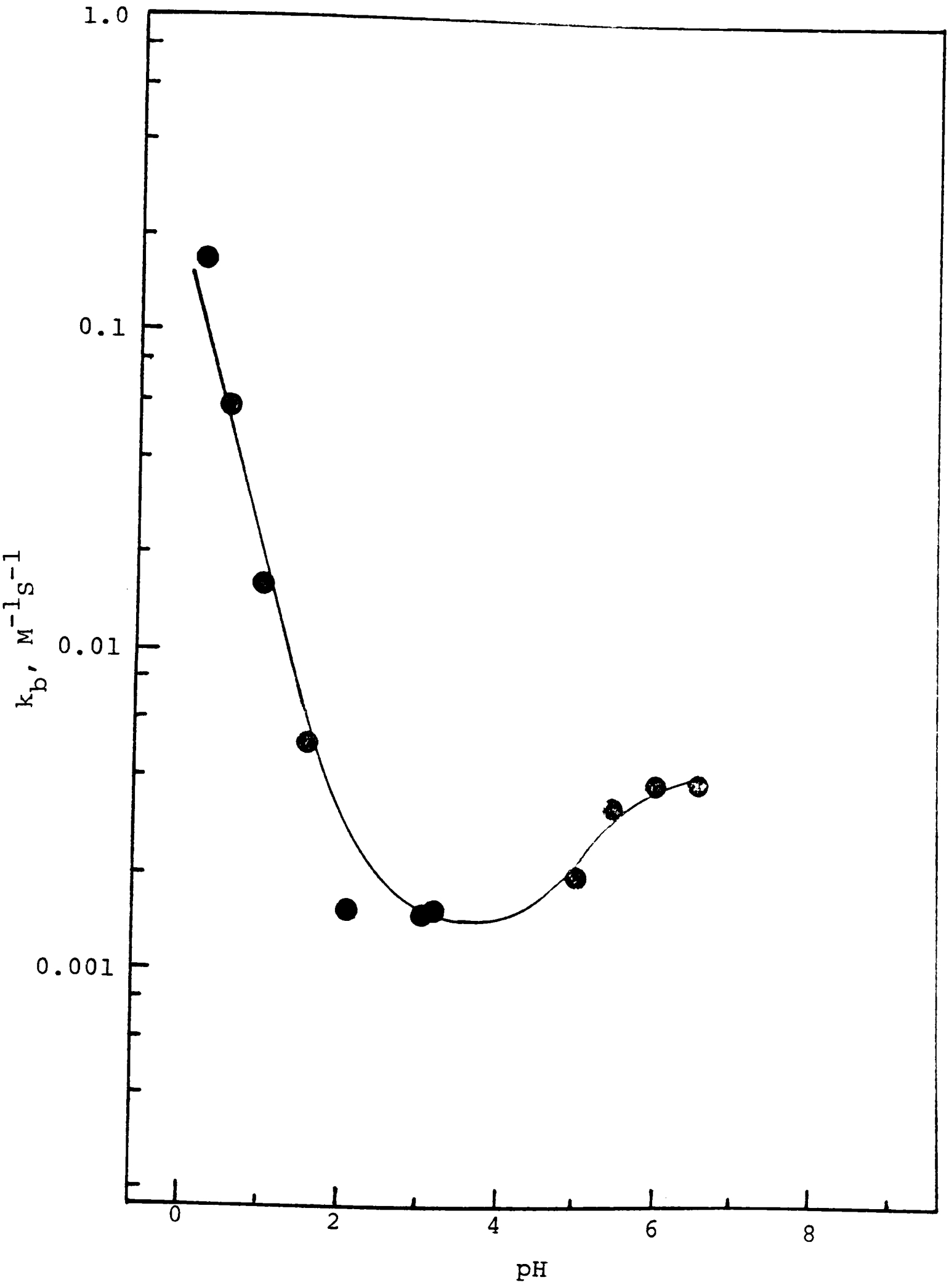

Figure 7

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APPENDIX

Preparation of Perchloric Acid and Buffers in 60% Dioxane

In all cases freshly prepared 60% dioxane (v/v), doubly distilled water and standard sodium hydroxide solution (Fisher, 1 N) were used. The pH of aqueous solution were determined using Radiometer Copenhagen PHM 62 standard pH meter. Concentration of acid used, [HA], or pH of solutions were calculated using the following equation:

$$pK = pH - \log[A]/[HA]$$

Preparation of Standard Perchloric Acid

1. Preparation of 6 N perchloric acid

Concentrated perchloric acid (Baker, 71% by wt.), 24 ml, was pipetted into a 50 ml volumetric flask, and then filled to the mark with doubly distilled water.

2. Preparation of 0.5 N perchloric acid.

Concentration perchloric acid (Baker, 71% by wt.), 4 ml, was pipetted into a 100 ml volumetric flask, and then filled to the mark with doubly distilled water.

3. Preparation of 0.1 N perchloric acid

The prepared 0.5 N perchloric acid, 20 ml, was pipetted into a 100 ml volumetric flask, and then filled to the mark with doubly distilled water.

Preparation of Perchloric Acid in 60% Dioxane

The correct amount of the prepared standard perchloric acid of the proper concentration was pipetted into a appropriate volumetric flask. A sufficient amount of pure dioxane was added to make the solution contain 60% dioxane, and the solution was then made up to the mark with 60% dioxane.

Preparation of Aqueous Buffers

The correct amount of the carboxylic acid was carefully weighed into a volumetric flask. An appropriate amount of sodium hydroxide solution was added so that the buffer had the desired acid to salt ratio. The solution was then made up to the mark with doubly distilled water.

Preparation of Buffers in 60% Dioxane

The correct amount of the proper aqueous buffer was pipetted into an appropriate volumetric flask. A sufficient amount of pure dioxane was added to make the solution contain 60% dioxane, and the solution was then made up to the mark with 60% dioxane.

TABLE IV

pH of the Carboxylate Buffers

Buffer	Acid : Salt	pH in Water	pH in 60% Dioxane[a]
$CF_3COOF-CF_3COO^-Na^+$	1 : 1	0.25[b]	2.8
	0.5 : 1	0.55[b]	3.1
	0.25 : 1	0.85[b]	3.4
$CHCl_2COOH-CHCl_2COO^-Na^+$	0.38 : 1	1.95	4.5
$CH_2ClCOOH-CH_2ClCOO^-Na^+$	3.8 : 1	2.3	4.9
	1.1 : 1	2.8	5.4
$HCOOH-HCOO^-Na^+$	1.3 : 1	3.65	5.9
	0.3 : 1	4.25	6.5
$CH_3COOH-CH_3COO^-Na^+$[c]	1 : 1	4.76	7.4
	0.2 : 1	5.46	8.1

[a] pH's for various carboxylic acids were derived from the measurements either in this laboratory[21] or others[22], of pK_a, is the variation of K_a of each of the carboxylic acid when transferred from water to 60% dioxane as a solvent.

[b] pH was obtained by calculation.

[c] Buffer was prepared with proper amount of acid and its salt.