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A kinetic study of the aminolysis of thioesters related to coenzyme A

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A KINETIC STUDY OF THE AMINOLYSIS OF THIOESTERS RELATED TO COENZYME A

DAVID J. McCONNELL

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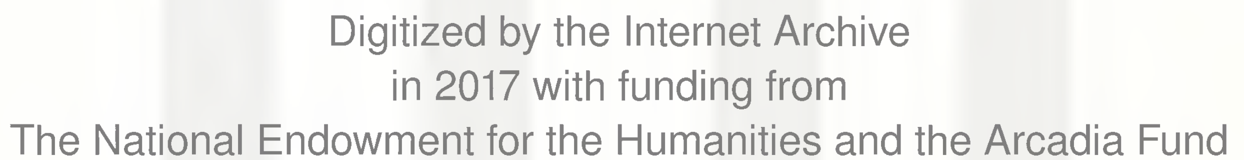
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A KINETIC STUDY OF THE AMINOLYSIS
OF THIOESTERS RELATED TO COENZYME A

by

David J. McConnell
Cornell University, 1955-1958

A Thesis
Presented to the Faculty
of the Yale University School of Medicine
in Partial Fulfillment of the Requirements
for the Degree of
Doctor of Medicine

Department of Pharmacology
1962

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The author would like to thank Dr. Henry G. Mautner for his generous help and advice. He would also like to thank Dr. Wolfgang H.H. Günther for his many helpful suggestions.

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Introduction

Many analogs of biologically active compounds have been made in a search for drugs which would be effective against neoplastic disease. The theoretic purpose of a given analog or "anti-metabolite" is to take advantage of certain properties of tumor cells such as requiring more or having less of a given compound than normal cells. One compound, which has been studied extensively, but, for which no good anti-metabolite has yet been devised, is coenzyme A.

In 1948, Potter and his group[1,2] found that homogenates of certain tumors (Flexner-Jobling and Walker 256) showed minimal conversion of oxaloacetic acid to citric acid. Citrate did accumulate in normal liver and kidney homogenates, but not in brain and heart. However, there was appreciable O_2 uptake and keto acid disappearance in brain and heart, but only a negligible amount in tumor homogenates. No attempt was made to identify the defect in tumor tissue.

In 1950, Higgins and his group[3] found that, compared with normal liver tissue, the CoA and pantothenic acid content was low in Flexner-Jobling and Walker tumors, the Jensen sarcoma, and azo dye induced rat liver tumors. The majority of each factor in normal liver homogenates was found in the mitochondria as opposed to the supernatent fraction in tumor homogenates. Thus, the poor conversion of oxaloacetic acid to citric acid and the low oxygen uptake in tumors observed

by Potter might be caused by the low CoA content of these tissues and the inadequate formation of acetyl-CoA. In the papers cited above, non-regenerating normal tissue was used. The studies would have been more conclusive had actively regenerating normal tissue been compared with neoplastic tissue.

Emmelot and Bosch[4], in 1955, studied the rate of synthesis of cholesterol and fatty acids in a number of mouse ovarian granulosa and sarcomatoid tumors. They found that the CoA content of these tumors broadly paralleled their synthetic capacities _in vitro_. However, in two tumors a discrepancy between CoA content and synthetic capacity was found. Therefore, they felt that the level of CoA was not the rate limiting factor involved in this synthetic process.

In 1954, Biesele[5] reported a study on inhibition by 6-Mercaptopurine of embryonic skin, mouse sarcoma 180, other mouse tumors, and two human tumors. He found several physiologic purines, nucleosides, and nucleotides partially reversed the mitotic inhibition of sarcoma 180 by 6-Mercaptopurine. Of these a CoA preparation was found to be the most effective. As the CoA used was only 30% pure, it is possible that other metabolites antagonistic to 6-Mercaptopurine were also present in the preparation.

Bolton and Mandel[6], in 1957, studied the effects of 6-Mercaptopurine on _Escherichia coli_ B. In order of decreasing sensitivity they found inhibition of growth, acetate utilization, formate utilization, nucleic acid content, and protein and

lipid content. 6-Mercaptopurine was found to depress acetate utilization in the synthesis of protein and lipid without decreasing their content in the cell. This effect was readily reversed by the addition of purines (hypoxanthine, adenine or guanine). Since the flow of acetate carbon into lipid and protein takes largely independent pathways, it was felt that this particular inhibitory effect took place early in the stages of acetate utilization - possibly antagonizing the synthesis or utilization of a purine containing compound such as adenosine tri-phosphate or CoA. In this study, it is impossible to isolate the various functions studied from each other. For instance, one cannot say whether growth inhibition by 6-Mercaptopurine is secondary to depressed acetate utilization or *vice versa*, or whether the two functions are entirely independent. However, the authors did feel that the drug probably exerts its effect on one or more purine-containing co-factors - possibly CoA.

In view of the above work, it seems feasible that an anti-metabolite of CoA might be devised. Along this line, a number of pantothenic acid and pantetheine analogs have been studied.

Bird *et al.*[7], in 1955, summarized the work that had been done to date on pantothenic acid antagonists and reported on their own findings with ω- methyl pantothenic acid. Most of the previously studied pantothenic acid analogs

had been found to inhibit only microorganisms. Only one of them, ω methyl pantothenic acid, had been found by Drell and Dunn[8] to produce a pantothenate deficiency in mice. Shils[9] had also found that rats had a decreased ability to acetylate sulfanilamide when receiving ω methyl pantothenate. Bird and his group found that neither ω methyl pantothenate or ω methyl pantethine inhibited the growth of rats. They did find that ω methyl pantothenate and ω methyl pantethine tended to inhibit the utilization of pantothenate and pantethine respectively in bacteria. In light of these observations, it may be said that the work of Drell and Dunn was incorrect and that the observed "pantothenate deficiency" was caused by some other unknown factor. In view of this work, we can say that, to date, we do not have a good pantothenic acid antagonist which will significantly inhibit CoA activity in animals.

A number of attempts have also been made to synthesize a suitable pantetheine analog. Stewart, Cheldelin, and King[10], in 1955, reported the synthesis of a number of these. (See Table I) N - Pantoyl - 2 - aminoethanethiol (II), N-- Pantoyl-ethanolamine (III), and Bis (N - (pantoyl-norvalyl) - 2 - amino - ethyl) disulfide (IX) were unable to support growth of L. helveticus (a pantetheine requiring organism[11]). N - (Pantoylglycyl) - 2 - aminoethane - thiol (VI), N - (Pantoyl-alanyl) - 2 - aminoethanethiol (VII), and Bis (N - (γ-hydroxy butyrl) - β alanyl - 2 - aminoethyl) disulfide (VIII)

were able to support some growth of L. *helveticus*. Because of these findings, they postulated that the two amide linkages and their spacing in the pantetheine molecule were important for biologic activity - possibly as points of attachment to an enzyme protein. Substitution of a methyl group on the methylene carbon of the glycine analog (VI) to produce the alanine analog (VII) did not change its biologic activity. However, when a large group was substituted as in (IX) all activity was lost - again indicating the importance of the configuration of this portion of the molecule. The γ hydroxy butyric acid analogue (VIII) was also relatively inactive, which indicates the importance of the pantoic acid configuration. "Oxypantetheine" (N - Pantothenoyl - ethanolamine V) was found to be a competitive inhibitor of pantetheine in L. *helveticus* with an inhibition index of 100 to 200:1 at 50% inhibition. Surprisingly, it was also found that low concentrations of oxy-pantetheine had a synergistic effect with pantetheine in L. *fermenti*. It is difficult to arrive at any definite conclusion from these observations other than that the sulfur atom appears to be essential to the activity of pantetheine.

In 1955, Pierpont, *et al.*[12] tested the compounds shown Table II to see if they could support growth and CoA synthesis in various bacteria. Besides pantetheine, only pantothenoylglycine supported growth in L. *arabinosus* and this growth

occurred only after its hydrolysis to pantothenic acid. None of the compounds (pantothenoyl - L - glutamic acid and N - pantothenoyl - 2 - hydroxy ethyl amine were not tested) replaced pantetheine in L. helveticus. The fact that pantothenoyl - L - cysteine, dipantothenoyl - L - cystine, and pantetheine 4' phosphate did not support growth in L. arabinosus is somewhat surprising in view of the proposed synthetic pathway for CoA[13]. This observation was attributed to the failure of these compounds to enter the cell. In view of this problem, it is difficult to evaluate the significance of the fact that the other pantothenate derivatives also failed to support growth in this organism.

In 1959, Mautner and Günther[14], reported the synthesis of seleno-pantethine and its ability to completely replace pantethine in L. helveticus. Lam et al.[34] have suggested that selenite might be incorporated to form seleno - CoA and that this could be a functional analog of CoA. However, Mantner and Günther[37] have also found that selenopantethine inhibits the utilization of pantethine as a precursor of CoA in a pigeon liver system. These findings are interesting in view of the fact that Stewart et al.[10] found that oxypantetheine was less active than pantetheine in L. helveticus and could actually inhibit it.

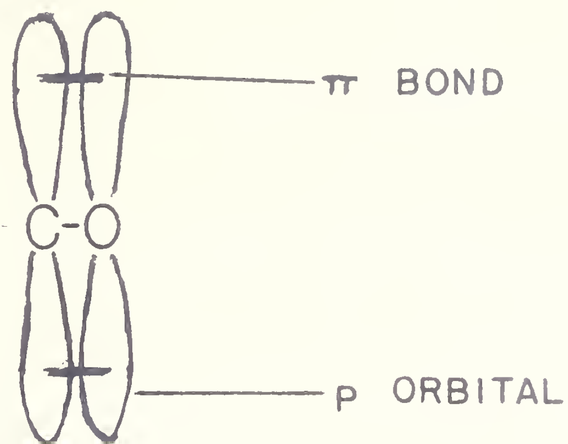
While, as yet, no suitable pantetheine antimetabolite has been found, the above work has shed light on some of the properties of the pantetheine molecule . Among other things

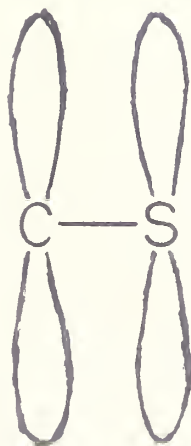
it has been shown that the sulfur atom in pantetheine is essential to the activity of CoA. Other workers have further elucidated the role of this atom in Coenzyme A.

In 1951, Lynen and Reichert[15], first identified acetylated CoA as a thioester. In 1960, Jaenicke and Lynen[19], reviewed the work and thinking that had been done on CoA and its properties up to that date. A number of model compounds of CoA had been made and tested in enzyme systems[19a]. In general these had a lower affinity for enzymes than CoA. It had been shown that the thioethanolamine structure and the acylated nitrogen are necessary for the activity of CoA. Jaenicke and Lynen theorized that the specific properties of acetyl-CoA are dependent on the sulfur atom[19b].

In any ester the carbonyl carbon acquires a positive charge activating it to nucleophilic attack. The carbonyl carbon of thioesters is more positive than that of oxy-esters because of the greater resistance of sulfur in the 2^- oxidation state to forming double bonds with carbon. This resistance is caused by the larger size of the sulfur atom which decreases the tendency of carbon and sulfur p orbitals to overlap to form a π bond[20,21]. (See Figure 1). This effect allows the electron cloud of the carbonyl π bond in thiocarbonyl groupings to be shifted more toward the carbonyl oxygen than in oxy-carbonyl groupings. Thus, the mesomeric shift of electrons toward the carbonyl carbon is less in thio

FIGURE I





than in oxy-esters and the carbonyl carbon of thio-esters is more positive than that of oxy-esters making it more prone to nucleophilic attack as in hydrolysis and aminolysis.

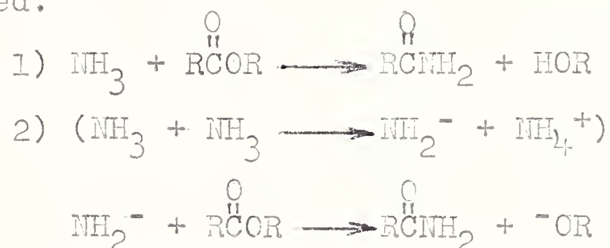
The strong positive charge on the carbonyl carbon also has other effects of importance in the reactions undergone by CoA esters. It induces a negative charge on the α carbon and a positive charge on the β carbon of an acyl residue. As a result of the α carbon's negative charge one gets electrophilic substitution at this point as in the formation of acetoacetyl CoA from acetyl CoA. The importance of the induced positive charge on the β carbon can be seen in fatty acid metabolism at the point where a water molecule is added to the α-β unsaturated bond of the fatty acid. The hydroxyl group attacks the positive β carbon while a proton is added to the negative α carbon. The alcohol group is subsequently oxidized to a ketone which is attacked by CoA splitting off acetyl CoA and setting up a shorter chain fatty acid - CoA on which the process will be repeated.

From the above work, it appears that CoA exerts its activity through its ability to form thioesters. In 1955, Basford and Huennekens[16] suggested that CoA might also exist as a thiazoline ring form. They identified four separate forms of CoA, all of which had enzymatic activity. One was a free thiol, two were disulfides, and one, which reacted with nitroprusside only after addition of cyanide, but did

react slowly with indophenol, was thought to represent a thiazoline ring form. Since the disulfides could be reduced to mercaptans, three of the four forms could easily form thioesters. Basford and Huennekens postulated that the thiazoline ring could react with acyl groups in several ways. The ring could open and the compound could then form a conventional thioester, or it might form an acyl-thiazoline compound or, possibly, an acyl-hydroxy thiazolidine compound. The acyl group could attach to either the sulfur or nitrogen of the ring system forming a "high energy onium" compound. Both forms are feasible in view of work by Stadtman and White[17] and Cantoni[18]. Stadtman and White, in 1953, reported the transfer of acetate from acetyl CoA to the nitrogen of imidazole to form a high energy bond. Cantoni, in 1953, reported evidence that "active" methionine exists as S - adenosylmethionine with the methyl group attached to a positively charged sulfur. In this case, a methyl group is attached to the sulfonium sulfur, but it is possible that an acyl group could also attach to sulfur in this state. It should also be noted that Diago and Reed[43] have synthesized 2 - acetyl - 3, 4 - dimethyl thiazolium iodide and have found it to be a highly active acetate donor. Thus, CoA - acyl compounds might exist as thiazoline derivatives as well as thioesters.

This possibility was one of the major stimuli for the present paper. Before going into this work, which involves a comparison of the aminolysis of two thioesters related to CoA, it would be well to review some of the work that has been done in the field of oxy- and thioesters.

In 1937, Betts and Hammett[30] published their study on the aminolysis of phenylacetic esters. They came to the conclusion that the following parallel reactions were involved.



1) $NH_3 + RC(=O)OR \longrightarrow RC(=O)NH_2 + HOR$

2) $(NH_3 + NH_3 \longrightarrow NH_2^- + NH_4^+)$

$NH_2^- + RC(=O)OR \longrightarrow RC(=O)NH_2 + {}^-OR$

Schaefgen[23], in 1948, compared the hydrolysis of ethylthioacetate with ethyl acetate. He found that, while the reaction rates for basic hydrolysis of ethylthioacetate and ethylacetate were about the same, ΔH (energy of activation) and log PZ (entropy of activation) for ethylthioacetate hydrolysis were both higher. Since a higher ΔH would hinder the reaction while a higher log PZ would favor it, the two effects neutralized each other in the overall rate. Schaefgen based his interpretation of these findings on the assumption that the C - S bond is less polarized than the C - O bond. In view of later work[19,20,21], which has been reviewed above, this assumption was incorrect. The proper explanation of these findings is probably similar

to that put forth by Rylander and Tarbell in 1950. Rylander and Tarbell[24] studied the hydrolysis of a number of oxy- and thioesters. They found that as the electron releasing ability of the thiol and alcohol group goes up, the energy of activation for the hydrolysis of the ester rises - more so for thioesters than oxy-esters.

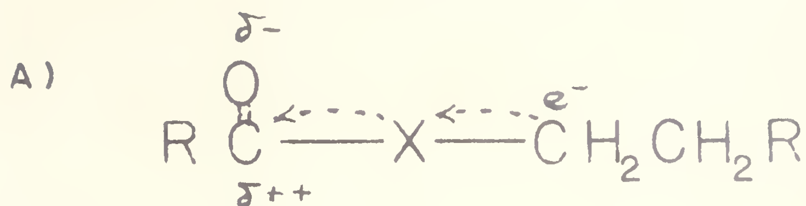
This observation probably reflects a decrease in positive charge at the carbonyl carbon with an increase in alcohol and thiol electron releasing ability. Rylander and Tarbell felt that the difference between thio- and oxy-esters was caused by the greater polarizability of the sulfur atom allowing a greater shift of negative charge through to the carbonyl carbon. (See Figure 2a).

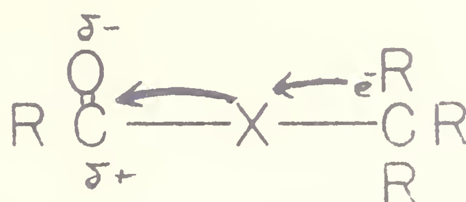
They also mentioned the possibility of a "hyperconjugative" mechanism as discussed by Crawford[27] which would also increase the electron density of the carbonyl carbon, but which would decrease as the electron releasing ability of the thiol or alcohol group increased. (See Figure 2b). They felt that especially in one of the compounds (iso butyl alcohol + thiol moiety) the high ΔH observed was caused by a large contribution through hyperconjugation.

The log PZ (entropy) factor for thioesters was uniformly higher than that for the corresponding oxy-esters. This observation could be secondary to the larger size of the sulfur atom and therefore the greater freedom of movement around the thioester bond. Another explanation, based on a suggestion

FIGURE 2

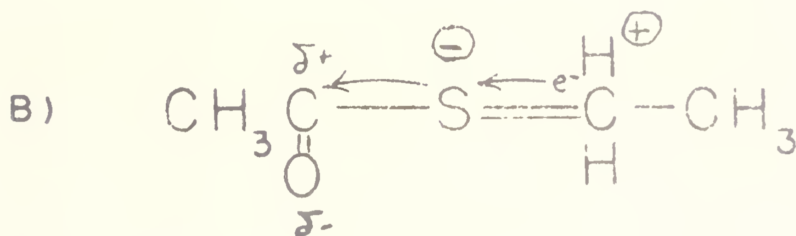
A)



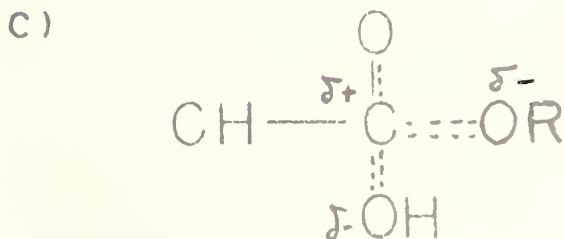


X = O OR S

B)



C)



by Dewar[25] is that thioesters would have a decreased tendency to enter a planar transition state during aminolysis, since as was noted above sulfur in the $^{-}2$ oxidation state resists the formation of double bonds. (See Figure 2c). Such a planar transition state would have a low log PZ factor.

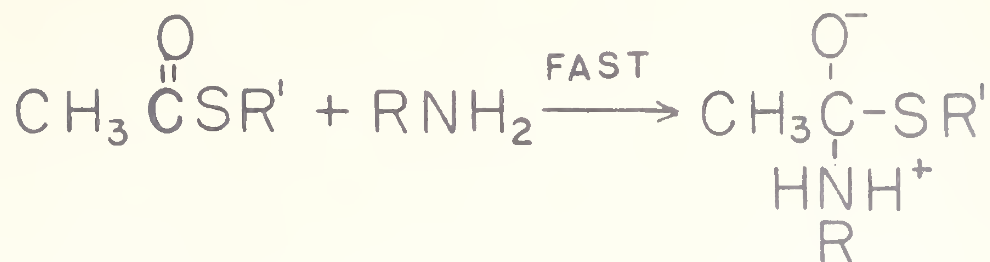
In 1953, Hawkins and Tarbell[28], compared the rates of aminolysis and hydrolysis of ethyl thioacetate and β - acetaminoethyl thioacetate using n - butyl amine in aqueous solution. This work was done in order to determine if the β - acetoamino group in the CoA molecule has an effect on its properties as a thioester. They found that the rates of hydrolysis and aminolysis for the two compounds were similar and concluded that, in this situation, the β - acetoamino group was not important in effecting the reaction. They found that the aminolysis obeyed the following equation:

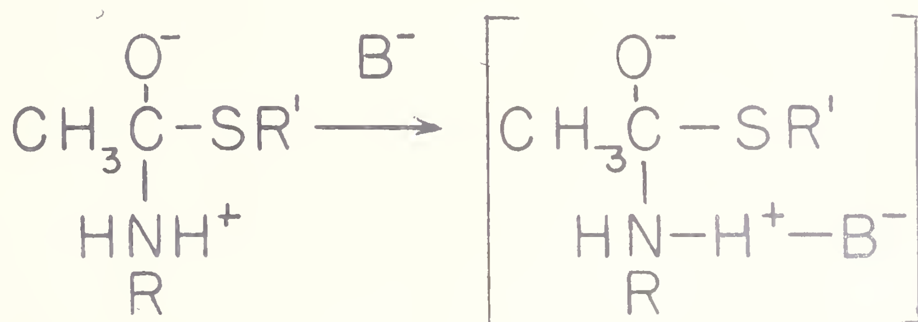
$$\frac{dE}{dt} = k_{obs} [\text{Ester}] = k_1 [\text{Ester}] [\text{RNH}_2] [\text{OH}^-] + k_2 [\text{Ester}] [\text{OH}^-]$$

Although other mechanisms would have fitted their results, they felt that the following was most likely. (See Figure 3). Hawkins and Tarbell were the first to suggest this general base catalysis as a mechanism for aminolysis and, although their theory was purely conjecture, subsequent work which will be noted below indicated they were correct.

The fact that, in all the work cited above, the aminolysis obeyed the rate equations only approximately was bothersome

FIGURE 3





and was clarified by Hawkins and Piskalnikow[29], in 1955. They studied the aminolysis and hydrolysis of α Naphthyl acetate by n - butyl amine in aqueous solution and varied the concentration of total amine and the ratio of RNH_3^+/OH^-. They found that the aminolysis obeyed the following equation.

$$k_{obs} = k_1 [RNH_2][OH^-] + k_2 [OH^-] + k_3 [RNH_3^+]$$

This finding indicates three parallel reactions - one, simple hydrolysis, two, base catalyzed aminolysis, and three, acid catalyzed aminolysis. The importance of each term would vary with pH.

It should be noted that the above terms are not the only ones which could fit the reaction. For instance $[RNH^-]$ could be substituted for $[RNH_2][OH^-]$ and $[RNH_2][H^+]$, for $[RNH_3^+]$. In other words, Hawkins and Piscalnikow did not specify the exact mechanism of the reaction.

The exact mechanism by which these reactions proceed in oxy-esters was further clarified in simultaneous papers by Bunnett and Davis[32] and Jencks and Carriuolo[33], in 1960. Bunnett and Davis studied the reaction of ethyl formate with n - butylamine in ethanol under different conditions by varying the concentrations of RNH_3^+, NaOEt, total concentration of amine, and ionic strength (LiCl). While in several places their data were rather imprecise, they felt their results were significant enough to disagree with the classic interpretation of Betts and Hammett[30], i.e. that a highly reactive

RNH⁻ ion is first formed which then attacks the ester. Instead the reaction is general base catalyzed. (See Figure 4). In view of their findings, other reaction mechanisms might be expected to occur, if the nature of the reactants were changed. For instance, if the nature of the alcohol group were changed to make it a better leaving group, one might expect the situation shown in Figure 5. In this reaction, the acid-catalyzed removal of the leaving group is no longer necessary (similar to the reaction mechanism proposed by Hawkins and Tarbell[28] for thioesters). As the attackings reagent became more acidic, one might expect the situation shown in Figure 6. Finally, if one had both a very acidic attacking reagent and a good leaving group, one might expect a mechanism such as that proposed by Betts and Hammett[30]. (See Figure 7).

Jencks and Carriuolo[33], studied the aminolysis of phenyl acetate by a number of different amines and came to similar conclusions.

Other work in the field of ester aminolysis and hydrolysis has been related to determining the effect on reaction rates of structural changes in the ester. In 1958, Heilbronn[35] reported a study on the rates of acid and base catalyzed hydrolysis of various thiocholine esters. Her results agreed with the work previously cited, although her interpretation based on the assumption that the C - O bond is more polarized

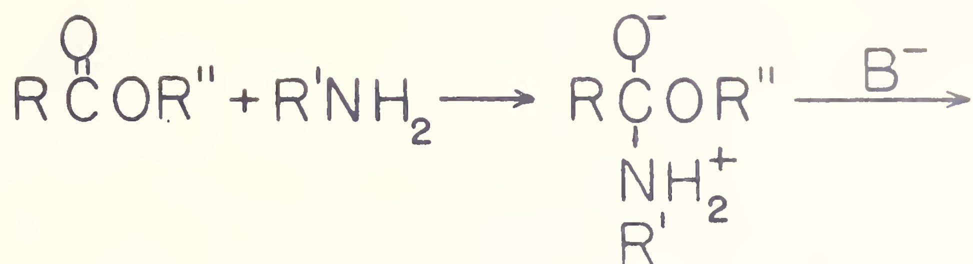
FIGURE 4

$$RC(=O)OR'' + R'NH_2 \rightleftharpoons \left[\underset{\text{I}}{RC(O^-)(OR'')NH_2^+R'} \rightleftharpoons \underset{\text{II}}{RC(OH)(OR'')NHR'} \right]$$

$$B^- + \text{I or II} \rightleftharpoons RC(O^-)(-OR'')NHR' + BH$$

$$RC(O^-)(NHR')-O(R'')\cdots H\cdots B \longrightarrow RC(=O)NHR' + R''OH + B^-$$

FIGURE 5



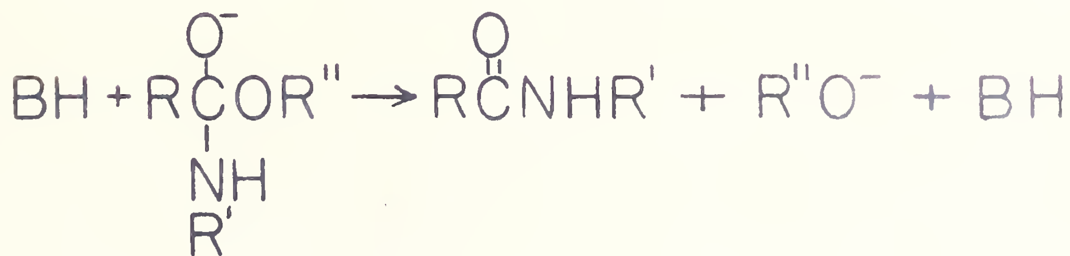
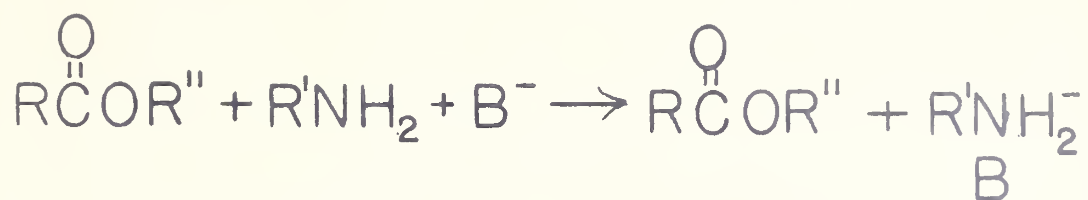


FIGURE 6



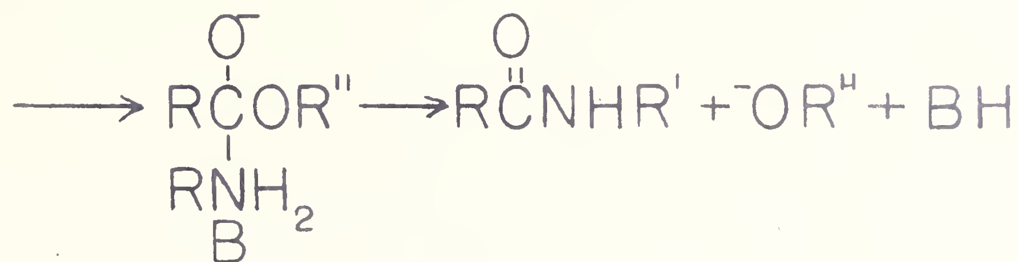
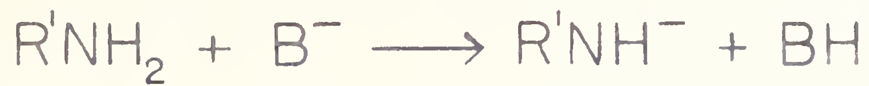
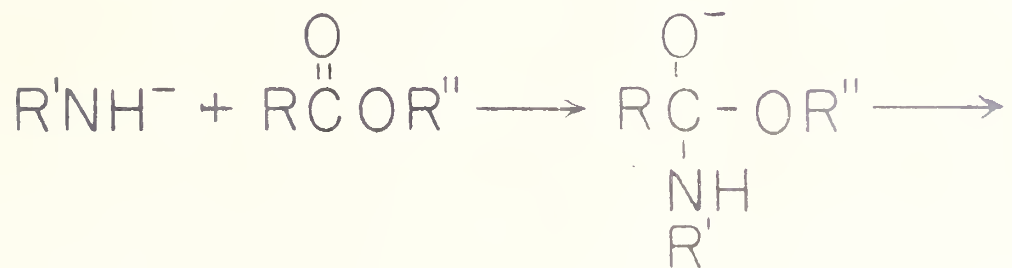


FIGURE 7





than the C - S bond seems incorrect in light of the work cited above[19,20,21]. An additional interesting finding in her paper was the observation that, as the electron donating ability of the acyl residue increased, the rate constant went down. This observation was interpreted as indicating a decrease in positive charge at the carbonyl carbon and a corresponding increase in energy of activation.

In 1960, Connors and Bender[22] reported a comparison of the alkaline hydrolysis and n - butyl aminolysis of ethyl - p - nitrobenzoate and ethyl - p - nitrothiobenzoate. The aminolysis of ethyl - p - nitrothiobenzoate followed the following general equation.

$$-\frac{dE}{dt} = k_1 [E][OH^-] + k_2 [E][RNH_2] + k_3 [E][RNH_2]^2 + k_4 [E][RNH_2][OH^-] + k_5 [E][RNH_2][RNH_3^+] + k_6 [E][RNH_2]$$

This equation had been previously suggested by the work of Bunnett and Davis[32], Jencks and Carriuolo[33], and Hawkins and Tarbell[28]. Under the conditions studied k_5 and k_6 equalled zero.

In 1961, Mautner and Günther[41] reported a preliminary study on the reactivity of thioacyl and selenoacyl analogs. In absolute ethanol, N, Se dibenzoyl selenocysteamine underwent more rapid n - butyl aminolysis than did N, S dibenzoyl cysteamine. In addition, reaction of N, Se dibenzoyl selenocysteamine with N - benzoyl cysteamine resulted in disappearance of the seleno-ester and appearance of the

thioester. The higher reactivity of the seleno-ester was attributed to greater polarization of the seleno-carbonyl bond. This interpretation is interesting in view of earlier studies showing an increase in polarization in passing from carbamyl, to thiocarbamyl to seleno-carbamyl analogs[38,39,40].

In 1956, Tarbell and Cameron[31] studied the hydrolysis and aminolysis of β (N - methyl acetamino) ethyl thioacetate, N, S diacetylaletheine, and γ -acetoamininopropyl thioacetate. Their purpose was to determine the effect on reaction rates of substitution around the acetoamino group of these model compounds of CoA. They found that the reaction rates obtained did not vary much. However, on checking their data, it is noted that the reaction rate for β - (N - methyl acetamino) ethyl thioacetate is slightly slower than that for the non-methylated compound-acetamino ethylthioacetate. (k_3 which represents the base catalyzed aminolysis was 1611 and 1827 respectively). Energy of activation for the aminolysis of the two esters did not appear to be significantly different. However, since the rate constants did appear to differ slightly, there may have been a steric effect in the reaction of the methylated compound which was relatively insignificant in the system studied.

This thought is interesting in light of the work of Basford and Huennekens[16] mentioned above in which a thiazoline ring was suggested as a possible form of CoA. The methyl

group on the nitrogen of β - (N - methyl acetamino) ethyl thioacetate would prevent the formation of a thiazoline ring. In light of the above studies, it was decided to study a similar system to that used by Tarbell and Cameron. In the present work, instead of the acetyl derivatives, N, S dibenzoyl cysteamine and N, S dibenzoyl N - methyl cysteamine were used and their rates of n - butyl aminolysis in absolute ethanol were compared. One might expect the benzoyl group to offer more steric hindrance to aminolysis than an acetyl group. This effect would then reduce the number of degrees of freedom, available to the reaction, thus reducing the entropy of activation and slowing the reaction rate. Since the overall number of degrees of freedom available to the reaction would be reduced, the further effect of the added methyl group in the N - methylated compound might become more noticeable in this situation.

Experimental

Synthesis. N, S dibenzoyl N - methyl cysteamine

Materials. N - methyl ethylene imine - Chemirad Corp.
East Brunswick, N.J.

Benzoyl Chloride - Allied Chemicals
General Chemical Division, New York

Preparation. N - methyl cysteamine is prepared by the method used by Tarbell and Cameron[31].

32 gms N - methyl ethylene imine in 60 cc dry methanol was added to approximately 30 gms H_2S in 60 cc dry methanol over a two hour period at dry-ice acetone temperature with constant stirring. H_2S was bubbled in throughout the addition. Following the addition, the solution was allowed to come to room temperature under an atmosphere of dry N_2. The methanol was then removed by vacuum at room temperature and the white crystalline product was washed with dry benzene. The product obtained was used immediately in the preparation of N, S dibenzoyl, N - methyl cysteamine. Yield was considered to be 100%.

Note: Identification of N - methyl cysteamine

During a repetition of the above synthesis a portion of the methanol solution obtained was set aside and placed under an atmosphere of dry N_2. The solvent was then removed by vacuum at room temperature. White crystals were deposited gradually and the product (1 gr) was filtered and washed with dry, cold heptane and placed in a vacuum desiccator.

M.P. - 40 - 45°C uncorrected Literature - 52 - 53°C[31]

N, S dibenzoyl N - methyl cysteamine

The N - methyl cysteamine obtained above was immediately dissolved in 100 ml dry benzene. Seventy-nine gms of benzoyl chloride dissolved in 200 ml of dry benzene was then added dropwise to the solution over a period of two hours with constant stirring at 0°C. A white gum formed. The mixture was then refluxed gently for 20 hours with the evolution of HCl. The solution was allowed to cool and the solvent was removed by vacuum leaving a clear oil which smelled of benzoyl chloride. This was then dissolved in petrol ether (b.p. 38.8° - 54.6°) and benzene (4:1) and on cooling on white solid crystallized out. This was then recrystallized from petrol ether and benzene.

Yield - 40 grs (24%) m.p. - 79° - 81°C

An analytic sample was prepared by recrystallization of 5 grs of the above from ligroine yielding white needles in clusters. m.p. - 82.5° - 84°C uncorrected

Analysis*	Theory	Found
C	68.20	68.13
H	5.72	5.78
N	4.68	4.74
S	10.71	10.51

Identification of Products. The following syntheses were carried out in order to identify the products obtained in the aminolysis runs described below.

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* Performed by Midwest Microlab Inc., Indianapolis, Indiana

N, n - butyl benzamide and N, N' dibenzoyl cystamine

Materials. n - butyl amine - Eastman Organic Chemicals, Rochester, New York

N, S dibenzoyl cysteamine - kindly donated by Dr. Günther

m.p. - 91.0° - 92.5°

lit. - 92.0° - 93.0°[36]

Preparation. Five ml of n - butyl amine was added to 3.0 gms of N, S dibenzoyl cysteamine in 20 ml of ethanol and the mixture was refluxed gently for 24 hours. The solvent was then removed by vacuum and the residual oil was dissolved in petrol ether (b.p. 38° - 58°) and ethyl ether and allowed to stand overnight. A white solid which had a positive disulfide test with nitroprusside and KCN crystallized out. This was then recrystallized from ether and ethanol yielding white crystals in the form of needles.

yield - 1.0 gm. (68%)
m.p. - 130° - 132°C uncorrected
lit. m.p. for N, N' dibenzoyl cystamine - 131.5° - 132.5°[38]

The mother liquor remaining from the crystallization of N, N' dibenzoyl cystamine was evaporated down leaving an oil which was dissolved in ligroine. On cooling, a white solid crystallized out, which on recrystallization yielded N, n - butyl benzamide.

yield - 0.9 grs. (48%)
m.p. - 38° - 39°C uncorrected
lit. - 41° - 42°[42]

N, n - butyl benzamide and N, N' dibenzoyl N, N' dimethyl cystamine

Materials. n - butyl amine - Eastman Organic Chemicals, Rochester, N.Y.

N, S dibenzoyl N - methyl cysteamine
prepared as noted above
m.p. - 79° - 81° uncorrected

Preparation. 4.9 gms of n - butyl amine in 50 ml of absolute ethanol was added to 20 gms of N, S dibenzoyl N methyl cysteamine dissolved in 75 ml of absolute ethanol and the mixture was refluxed gently for 24 hours. The ethanol was then removed by vacuum leaving a brown oil. Numerous attempts were made to obtain a crystalline product from this oil - all unsuccessful. The products were then isolated by column chromatography using an alumina column as follows:

Materials. alumina - 150 gms (Neutral - Brockmann grade I)
M. Woelm Eschwege, Germany

column diameter - 2.1 cm.

The alumina column was set up and washed with ether and the material was placed on the column. The column was first eluted with ½% ethanol in ether and a pale yellow oil (7.4 gms) came off which had a negative test for disulfide with KCN and nitroprusside. Recrystallization from ligroine yielded N n butyl benzamide.

yield - 5.1 gms (29%)
m.p. - 38° - 40° uncorrected lit. - 41° - 42°[42]

The column was then eluted with progressively greater concentrations of ethanol in ether until at 1% ethanol in ether a

yellow compound came off which gave a strongly positive disulfide test. All earlier and later fractions gave only weak or negative disulfide tests. This material was then concentrated to a clear yellow oil (5.0 gms). Subsequent chromatography of this material gave N, N' dibenzoyl N, N' dimethyl cystamine as a pale yellow oil.

yield - 4.1 gms (21%)

An analytically pure sample was prepared as follows. As the yellow contaminant appeared to be quite insoluable in pure ether, the disulfide, which was slightly soluable in ether, was partially dissolved in a large excess of ether. The ether was then decanted off and removed by vacuum at room temperature leaving a clear colorless oil (1.0 gm).

Analysis*	Calculated	Found
C	61.82	62.09
H	6.23	6.40
N	7.21	7.44
S	16.50	16.66

Kinetic Runs

Materials.

Beckmann DU Spectrophotometer
1 cm quartz cell cuvettes
Quaracell Products, New York, N.Y.
Automatic Cuvette Positioner Model 210
Guilford Instrument Laboratories Inc.
Oberlin, Ohio

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* Preformed by Schwarzhopf Microanalytical Laboratory, Woodside, N.Y.

Absorbance Indicator Model 220
Guilford Instrument Laboratories, Inc.
Optical Density Converter
Guilford Instrument Laboratories, Inc.
Constant Temperature Bath with Fisher Unitized Bath Control
Stopwatch
B-D Yale Tuberculin Syringe
Burton, Dickson, and Co.
Tele - Thermometer YSI Model 425C
Yellow Springs Instrument Co.
Yellow Springs, Ohio
n butyl amine - Eastman Organic Chemicals, Rochester, N.Y.
redistilled b.p. - 77.5° - 78° uncorrected
N, S dibenzoyl cysteamine - kindly donated by Dr. Günther
m.p. - 91.0° - 92.5°C uncorrected lit. 92.0° - 93.0°C[36]
N, S dibenzoyl, N methyl cysteamine prepared as noted above
m.p. - 82.5° - 84.0° uncorrected
N, n butyl benzamide - prepared as noted above
m.p. - 38° - 40° uncorrected
lit 41° - 42°[42]
N, N' dibenzoyl cystamine - prepared as noted above
m.p. - 130° - 132° uncorrected
lit 131.5° - 132.5° [36]
N, N' dibenzoyl N, N' dimethyl cystamine
analytically pure - prepared as noted above
Absolute Ethanol

Procedure. Solutions of ester in ethanol were prepared by appropriate dilution of a stock solution of a weighed amount of ester in ethanol. All runs were done in duplicate and were set up as follows:

	cuvette	ester	ethanol	amine
blank	# 1		3 cc	.1 or .2 cc
"reaction"	# 2	3 cc		.1 or .2 cc
"reaction"	# 3	3 cc		.1 or .2 cc
standard	# 4	3 cc	.1 or .2 cc	

All four cuvettes were allowed to come to equilibrium with the constant temperature bath along with the amine to be added to the "reaction" cuvettes. A run was started by injecting amine from a tuberculin syringe into cuvette # 2,

starting the stopwatch, and taking a reading. Three minutes later, amine was injected into the second "reaction" cuvette (# 3) and from this point both reactions were read simultaneously every 3 min. for the first 30 min. and every 6 min. for the second 30 minutes. Since the reaction rate was quite slow, the discrepancy of approximately 10 seconds in taking one reading after another did not affect the curves obtained and one had the advantage of making simultaneous duplicate runs. Each reading was checked against the standard and corrected, if this showed any fluctuation. The tuberculin syringe was calibrated with freshly distilled water and was found to deliver .1055 ± .0018 ml at the .1 ml mark and .2065 ± .0020 ml at the .2 ml mark.

Runs were measured at 265mμ (thiobenzoyl absorption peak for N, S dibenzoyl cysteamine and N, S dibenzoyl N - methyl cysteamine). The amount of absorption accounted for by products accumulating was negligible over the time the reactions were followed. As can be seen from the absorption spectra graphs (Graphs 1 & 2), the amount of absorption of solutions of N n butyl benzamide, N, N' dibenzoyl cystamine, and N, N' dibenzoyl, N, N' dimethyl cystamine in approximately equivalent amounts to 6.20 mg % of the two esters is small. These concentrations were never approached by the products over the time the reactions were followed.

Both esters were found to obey Beer's law over the range of concentrations measured (2.87×10^{-4} to 3.62×10^{-5} M). At 30°C E_{max} for N, S dibenzoyl, N methyl cysteamine is 8561, for N, S dibenzoyl cysteamine it is 9538. A tendency for absorbance to drift downward with increase in temperature was noted. Absorbance of 6.20 mg % N, S dibenzoyl N-methyl cysteamine at 3.6°C = 1.750, at 40.1°C = 1.664. Absorbance 2.5 mg % N, S dibenzoyl cysteamine at 3.6°C = 904, at 39.9°= 835. This tendency did not affect the results of the kinetic runs, as any given run was at a constant temperature and therefore all values for that run were proportionally higher or lower and the rate constant was not affected.

Results

Sample kinetic runs for the n - butyl aminolysis of N, S dibenzoyl cysteamine and N, S dibenzoyl N-methyl cysteamine are shown on Graph 3 and 4. Tables III and IV give the averages of the results from the various kinetic runs made. All the averages recorded are obtained from results which were observed to agree with each other within the experimental error. The standard deviation and ~~standard error~~ AVERAGE DEVIATION which could be expected in making this type of kinetic run were calculated from 8 runs of .1 ml n - butyl amine with 6.20 mg % N, S dibenzoyl, N - methyl cysteamine at approximately 30°C (29.3° ± .4°C) as shown in Table V. As can be seen in Tables III and IV, runs were made at several different concentrations of ester and values of k_{obs} for these runs were found not to vary significantly from each other with one exception. At low concentrations of N, S dibenzoyl cysteamine the k_{obs} was found to be significantly lower than at the higher concentrations. No explanation was apparent for this observation as values for k_{obs} obtained with widely varying higher concentrations did not vary significantly. With this exception, the above findings indicate that both reactions are 1st order in ester.

When concentration of amine was doubled, k_{obs} was found to more than double. To obtain the order of reaction in amine, k_{obs} for the aminolysis of both esters at high and low

concentrations of amine was divided by $[RNH_2]$, $[RNH_2]^{3/2}$ and $[RNH_2]^2$. This procedure yields the corresponding 1st order, 3/2 order, and 2nd order rate constants in amine. As can be seen in Table VI, both reactions have 3/2 order rate constants in amine which agree quite closely between .346 M and .653 M amine. However, the values for the higher concentrations of amine are both slightly up. This observation could be caused by either a rate constant that was slightly higher than 3/2 order in amine or an effect caused by increasing ionic strength of the solution with increasing concentration of amine. The second explanation seems more likely in light of the work by Bunnett and Davis[32] showing an increase in aminolysis rate constants with the addition of lithium chloride to solution. Thus, we can say with a reasonable degree of assurity that the reactions studied are 1st order in ester and 3/2 order in amine.

Energies of activation (ΔH^*) were calculated from the slope of Arrhenius plots (Graphs 5 and 6)

$$-\Delta H^* = \frac{2.303\ R\ (\log k_1 - \log k_2)}{\frac{1}{T_1} - \frac{1}{T_2}}$$

ΔH^* for the n - butyl aminolysis of N, S dibenzoyl N methyl cysteamine was found to be 9416 cal/mole. For N, S dibenzoyl cysteamine it was found to be 9455 calories/mole. These values were felt not to differ significantly.

Entropy of activation (ΔS^*) for the two reactions was

calculated from the formula.

$$\Delta S^* = 2.303\ R\ (\log k_{obs} - \log \frac{kT}{h}) + \frac{\Delta H^*}{T}$$

which is a form of the equation

$$k_{obs} = \left(\frac{kT}{h}\right) e^{\frac{\Delta S^*}{R}} e^{\frac{-\Delta H^*}{RT}}$$ [27]

For the n - butyl aminolysis of N, S dibenzoyl cysteamine, $\Delta S^* = -48.48$ cal/deg-mole. For N, S dibenzoyl N-methyl cysteamine, $\Delta S^* = -49.78$ cal/deg-mole.

The difference between the two entropies of activation can be calculated from the following formula, which eliminates the need for using any variables other than k_{obs} providing ΔH^* and T for the two reactions do not vary significantly.

$$\Delta S_1^* - \Delta S_2^* = 2.303\ R\ (\log k_1 - \log k_2)$$

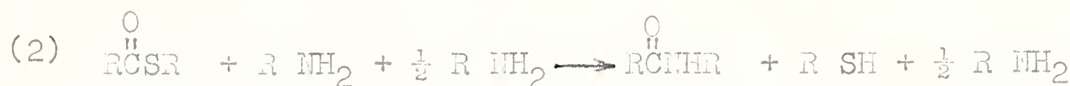
Letting ΔS_1^* represent ΔS^* for N, S dibenzoyl cysteamine and ΔS_2^* represent ΔS^* for N, S dibenzoyl N - methyl cysteamine, $(\Delta S_1^* - \Delta S_2^*)$ is found to equal + 1.28 cal/deg-mole which agrees very well with the difference found between the calculated values of ΔS^* for the two reactions (+ 1.30 cal/deg-mole). This finding indicates that the difference in rate between the two reactions is entirely caused by the difference in entropy of activation.

Discussion

The data summarized above indicate that the n - butyl aminolyses of N, S dibenzoyl cysteamine and N, S dibenzoyl N-methyl cysteamine are 1st order in ester, 3/2 order in amine and 5/2 order overall. This is equivalent to the reaction

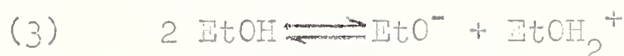
$$(1)\quad R\overset{O}{\overset{\|}{C}}SR' + 3/2\ R\ NH_2 \longrightarrow R\overset{O}{\overset{\|}{C}}NHR + R\ SH + \tfrac{1}{2}\ R\ NH_2$$

or



$$(2)\quad R\overset{O}{\overset{\|}{C}}SR + R\ NH_2 + \tfrac{1}{2}\ R\ NH_2 \longrightarrow R\overset{O}{\overset{\|}{C}}NHR + R\ SH + \tfrac{1}{2}\ R\ NH_2$$

In equation 2, the term $\frac{1}{2}\ RNH_2$ is obviously catalytic. The question is - what does it represent? The following equilibria are present in the solutions studied.



$$(3)\quad 2\ EtOH \rightleftharpoons EtO^- + EtOH_2^+$$

$$(4)\quad 2\ RNH_2 \rightleftharpoons RNH^- + RNH_3^+$$

$$(5)\quad RNH_2 + EtOH \rightleftharpoons RNH_3 + EtO^-$$

$$(6)\quad RNH_2 + EtOH \rightleftharpoons RNH^- + EtOH_2^+$$

One or more of the above terms should be equivalent to the catalytic term in amine, $\frac{1}{2}\ RNH_2$. Since ethanol is weakly dissociated compared to amine, we can assume equation (3) makes a negligible contribution to the catalytic term. Equations (5) and (6) would require catalytic terms 1st order in amine. Combinations of equations (4), (5) and (6) would require catalytic terms between $\frac{1}{2}$ and 1st order in amine. Catalysis by undissociated amine would provide a catalytic term 1st order in amine. Base catalysis to form

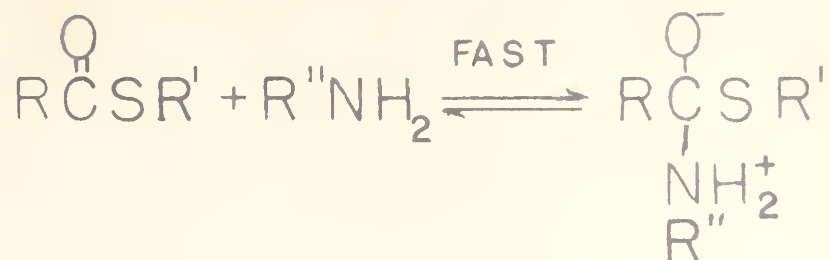
an amide ion which then attacked the ester would provide a reaction 1st order in amine over-all and zero order in catalytic amine. One might postulate that a combination of this reaction of zero order with one of those previously mentioned of order higher than $\frac{1}{2}$ would give an over-all reaction $\frac{1}{2}$ order in catalytic amine. This situation is unlikely in light of the work by Bunnett and Davis[32] which showed that one could expect base catalyzed attack by an amide ion, if one had a very acidic attacking reagent. This is not the situation in the reaction studied. Equation (4) does allow a catalysis term $\frac{1}{2}$ order in amine. It has been shown by Bunnett and Davis[32] that RNH_3^+ actually retards aminolysis. Therefore it appears that RNH^- is the most likely catalytic agent.

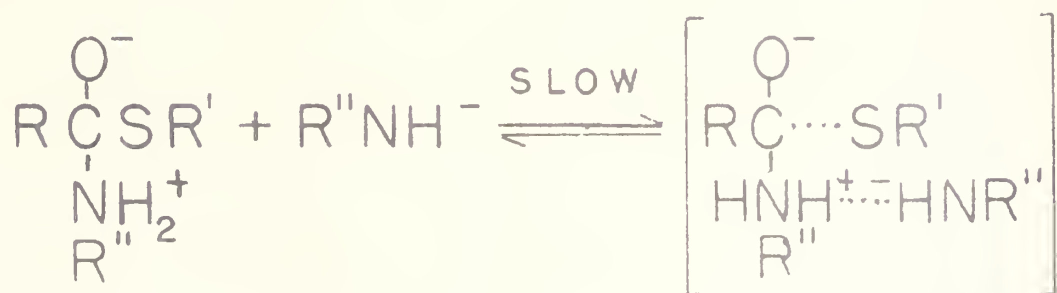
Since the amount of RNH^- in solution must be small in comparison with RNH_2, the formation and attack of RNH^- on a complex of RNH_2 and ester would have to be the rate-limiting step, if the over-all reaction in amine is to be 3/2 order. (See Figure 8).

This is equivalent to the equation

$$\overset{O}{\overset{\|}{RCSR}} + R\,NH_2 + R\,NH^- \longrightarrow \overset{O}{\overset{\|}{RCNHR}} + R\,SH + R\,NH^-$$

FIGURE 8





Assuming that the addition of RNH^- is the rate limiting step this equation is equivalent to equation (2) noted above.

The above mechanism is similar to those postulated for general base catalysis of esters by Hawkins and Tarbell[28], Jencks and Carriulo[33] and Bunnett and Davis[32]. It is interesting to note that this mechanism does not require additional acid catalysis to facilitate removal of the thio moiety. According to Bunnett and Davis[32], this acid catalysis is required in aminolysis of oxy-esters. However, they predicted that as one obtained a better leaving group such as RS^-, the need for the additional acid catalysis would disappear. Further evidence for this mechanism is presented below in a discussion of the entropies of activation for the two reactions studied.

It was felt that all evidence obtained pointed away from the formation of a thizaoline ring form by N, S dibenzoyl cysteamine such as might have been expected from the work of Basford and Huennekens[16]. Both esters had similar thioester absorption peaks at 265 mμ and reacted in similar ways with energies of activation which were felt to be identical.

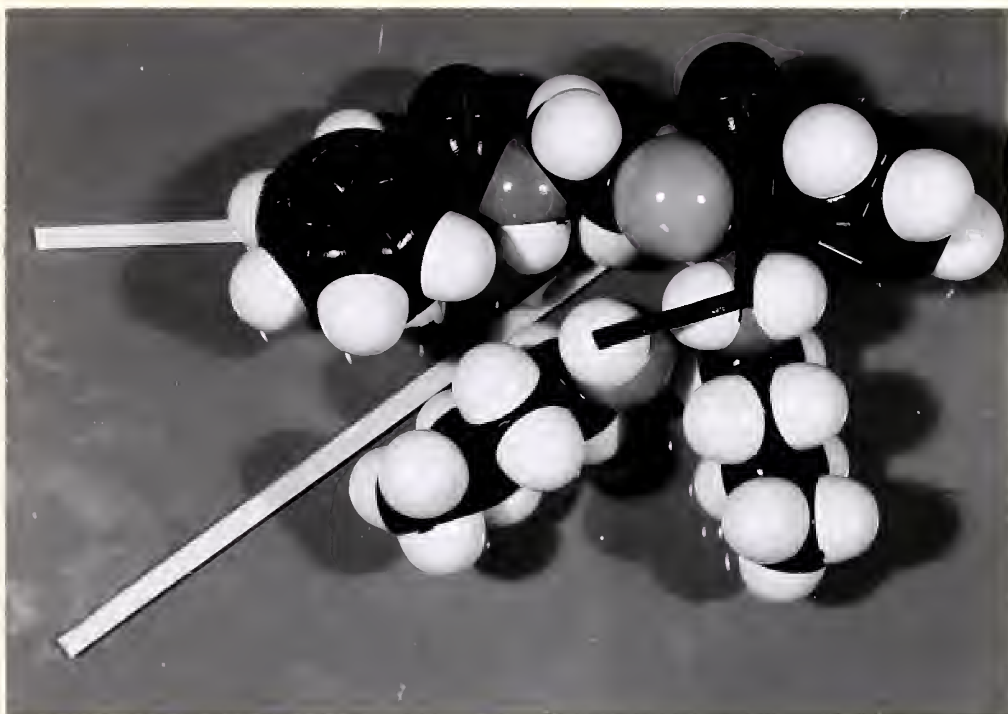
N, S dibenzoyl N-methyl cysteamine did react more slowly, but this was felt to be secondary to a moderate steric hindrance by the methyl group. This hindrance was measured through a determination of the entropies of activation for the two reactions.

Both entropies of activation were negative indicating a loss in degrees of freedom in the formation of the activated complex -- i.e. the reacting molecules went from a state of comparative freedom to one of comparative rigidity. However, the entropy of activation for the N - methylated compound was more negative than that for the non-methylated one. Evidently, the methyl group offered a steric hindrance to the reactants in the aminolysis of the N -- methylated compound forcing them to assume a more rigid complex. The only mechanisms in which such a hindrance might be found are those involving general base catalysis, in this case, according to the reasoning above, catalysis by n - butyl amide ion. Direct attack by amine or amide ion would not be hindered.

Models of the molecues involved were made. It was shown that there is indeed more hindrance of general base catalysis by amide ion in N, S dibenzoyl N -- methyl cysteamine than in N, S dibenzoyl cysteamine. One situation demonstrating the steric hindrance of the methyl group is shown in Figs. 9 and 10. This steric hindrance is a subtle effect. Nevertheless, since the energies of activation for the two reactions do not

FIGURE 9

N,S DIBENZOYL CYSTEAMINE
+ N-BUTYL AMINE



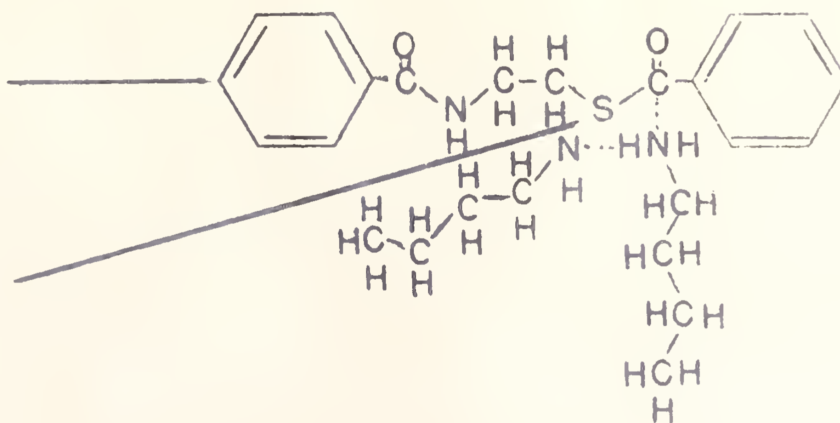
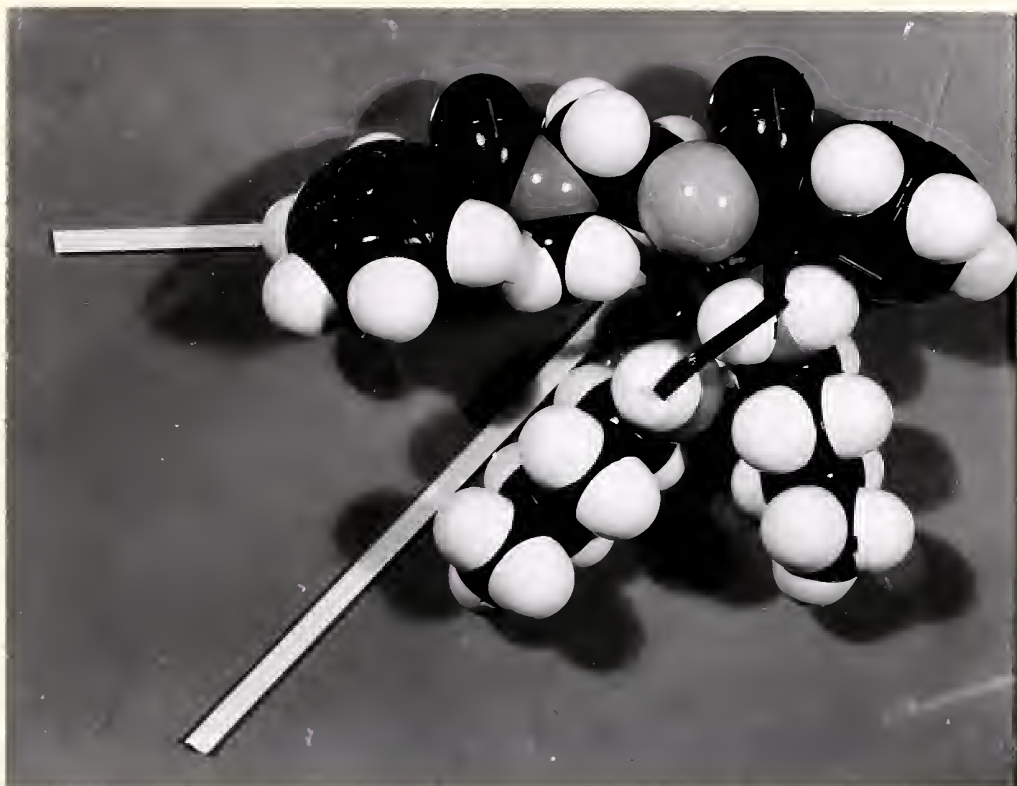
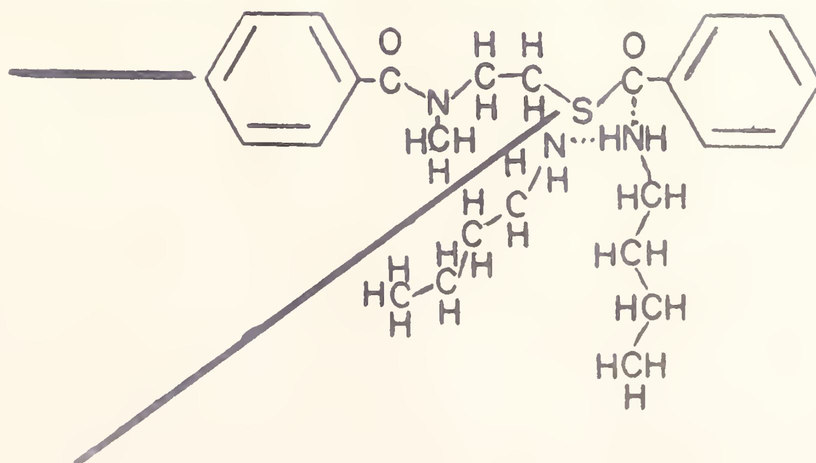


FIGURE 10

N,S DIBENZOYL N-METHYL CYSTEAMINE
+ n-BUTYL AMINE





differ significantly, it is solely responsible for an almost two-fold increase in the rate of n - butyl aminolysis of N, S dibenzoyl cysteamine over N, S dibenzoyl N - methyl cysteamine.

The results obtained disagree somewhat with the observations of Tarbell and Cameron[31] on N, S diacetyl cysteamine and N, S diacetyl N - methyl cysteamine, although their results do show a slight decrease in the rate of the N - methylated ester. As postulated in the introduction, it was felt that the more marked effect observed in the present work was caused by the presence of the benzoyl group instead of the acetyl group. The presence of the bulky benzoyl reduces the over-all number of degrees of freedom to the point where the effect of the methyl group becomes noticeable.

Because of this effect, one can postulate that N - methyl pantetheine might be a suitable anti-metabolite. One might expect it to be metabolized to N - methyl CoA which would then compete with normal CoA for enzyme receptor sites, since their over-all configurations would be similar. However, once attached to the receptor site N - methyl CoA might be expected to be rather ineffective since the steric hindrance offered by the methyl group would hinder efficient co-operation between the enzyme protein and the cysteamine portion of the co-enzyme. Of course, this reasoning is purely hypothetical, but hopefully, it will stimulate further work toward the synthesis and study of N - methyl pantetheine.

Summary

N, S dibenzoyl N-methyl cysteamine and N, N' dibenzoyl, N, N' dimethyl cystamine were synthesized. The n - butyl aminolyses of N, S dibenzoyl N-methyl cysteamine and N, S dibenzoyl cysteamine were studied and compared. It was felt that both reactions were general base catalyzed, in this case, by n - butyl amide ion. The slower rate for the n - butyl aminolysis of N, S dibenzoyl N-methyl cysteamine was attributed to a more negative entropy of activation for this reaction caused by steric hindrance from the methyl group.

Table I

see Stewart (ref 10)

	Compound	Formula
I	Pantetheine	$HOCH_2C(CH_3)_2CHOHCONHCH_2CH_2CONHCH_2CH_2SH$
II	N-Pantoyl-2-amino ethanthiol	$HOCH_2C(CH_3)_2CHOHCONHCH_2CH_2SH$
III	N-Pantoyl ethanolamine	$HOCH_2C(CH_3)_2CHOHCONHCH_2CH_2OH$
IV	Pantothenoyltaurine Sodium Salt	$HOCH_2C(CH_3)_2CHOHCONHCH_2CH_2CONHCH_2CH_2SO_3Na$
V	N-Pantothenoyl ethanolamine	$HOCH_2C(CH_3)_2CHOHCONHCH_2CH_2CONHCH_2CH_2OH$
VI	N-(Pantoylglycyl)-2 aminoethanethiol	$HOCH_2C(CH_3)_3CHOHCONHCH_2CONHCH_2CH_2SH$
VII	N-(Pantoylalanyl)-2 aminoethanethiol	$HOCH_2C(CH_3)_2CHOHCONHCH(CH_3)CONHCH_2CH_2SH$
VIII	Bis (N-(γ-hydroxy-butryl)-β alanyl-2 aminoethyl)disulfide	$(HOCH_2CH_2CH_2CONHCH_2CH_2CONHCH_2CH_2S-)_2$
IX	Bis (N·(pantoyl-norvalyl)-2-aminoethyl)disulfide	$(HOCH_2C(CH_3)_2CHOHCONHCH(C_3H_7)CONHCH_2CH_2S^-)_2$

Table II

see Pierpont (ref 12)

General formula of pantothenoyl peptides:

$HOCH_2C(CH_3)_2CHOHCONHCH_2CH_2COR$

Compound	R
Pantetheine	$-NHCH_2CH_2SH$
Pantothenoylcysteine	$-NHCH(CO_2H)CH_2SH$
Pantothenoyl-2-hydroxy-ethylamine	$-NHCH_2CH_2OH$
Pantothenoylamino-acetaldehyde	$-NHCH_2CHO$
Pantothenoylglyine	$-NHCH_2CO_2H$
Pantothenoyl-α-alanine	$-NHCH(CH_3)CO_2H$
Pantothenoyl-β-alanine	$-NHCH_2CH_2CO_2H$
Pantothenamide	$-NH_2$
Pantothenoylglutamic acid	$-NHCH(CO_2H)CH_2CH_2CO_2H$
Pantothenoylserine	$-NHCH(CO_2H)CH_2OH$

Table III

Reaction Rates for

N, S Dibenzoyl Cysteamine + n Butyl Amine

Temperature	[Ester]	[Amine]	avg k_{obs} (sec.$^{-1}$) x 10^{-5}
29.8°	2.5 mg %	.346M	2.378
29.2°	6.20 mg %	.346M	2.419
30.3°	1.25 mg %	.346M	unreliable*
30.3°	6.20 mg %	.653M	6.867
30.8°	1.60 mg %	.653M	5.063
3.6°	2.5 mg %	.346M	.5317
36.3°	2.5 mg %	.346M	3.280

* results of multiple runs were very low and erratic and did not agree within experimental error.

Table IV

Reaction Rates for

N, S Dibenzoyl N-Methyl Cysteamine + n Butyl Amine

Temperature	[Ester]	[Amine]	$k_{obs} \times 10^5$ (sec.$^{-1}$) (avg)
29.3°	6.20 mg %	.346M	1.259
29.9°	1.61 mg %	.346M	1.378
29.0°	4.30 mg %	.346M	1.280
29.2°	8.60 mg %	.346M	1.282
29.7°	1.61 mg %	.653M	3.577
4.2°	6.20 mg %	.346M	.3557
40.1°	6.20 mg %	.346M	1.973

Table V

Determination of error

series of 8 runs of 6.20 mg % N, S dibenzoyl N-methyl cysteamine + 1 ml amine at 29.3° ± .4°C. (Runs were done at widely different times and presumably take into account a certain amount of machine fluctuation)

	k_{obs} (sec.$^{-1}$)
# 1	1.126×10^{-5}
# 2	1.162×10^{-5}
# 3	1.195×10^{-5}
# 4	1.228×10^{-5}
# 5	1.329×10^{-5}
# 6	1.192×10^{-5}
# 7	1.439×10^{-5}
# 8	1.402×10^{-5}
avg	$1.259 \times 10^{-}$

average deviation ± .098 (± 7.8%)

standard deviation ± .116 (± 9.2%)

Table VI

Determination of order of rate constant in amine

For the reaction of N, S dibenzoyl N - methyl cysteamine with n butyl amine

$[RNH_2]$	k_{obs} ($sec.^{-1}$)	$\frac{k_{obs}}{[RNH_2]}$	$\frac{k_{obs}}{[RNH_2]^{3/2}}$	$\frac{k_{obs}}{[RNH_2]^2}$
.346M	1.259×10^{-5}	3.64×10^{-5}	6.12×10^{-5}	10.58×10^{-5}
.653M	3.577×10^{-5}	5.38×10^{-5}	6.78×10^{-5}	8.27×10^{-5}

For the reaction of N, S dibenzoyl cysteamine with n - butyl amine

$[RNH_2]$	k_{obs} ($sec.^{-1}$)	$\frac{k_{obs}}{[RNH_2]}$	$\frac{k_{obs}}{[RNH_2]^{3/2}}$	$\frac{k_{obs}}{[RNH_2]^2}$
.346M	2.400×10^{-5}	6.94×10^{-5}	11.65×10^{-5}	20.1×10^{-5}
.653M	6.867×10^{-5}	10.51×10^{-5}	12.98×10^{-5}	16.25×10^{-5}

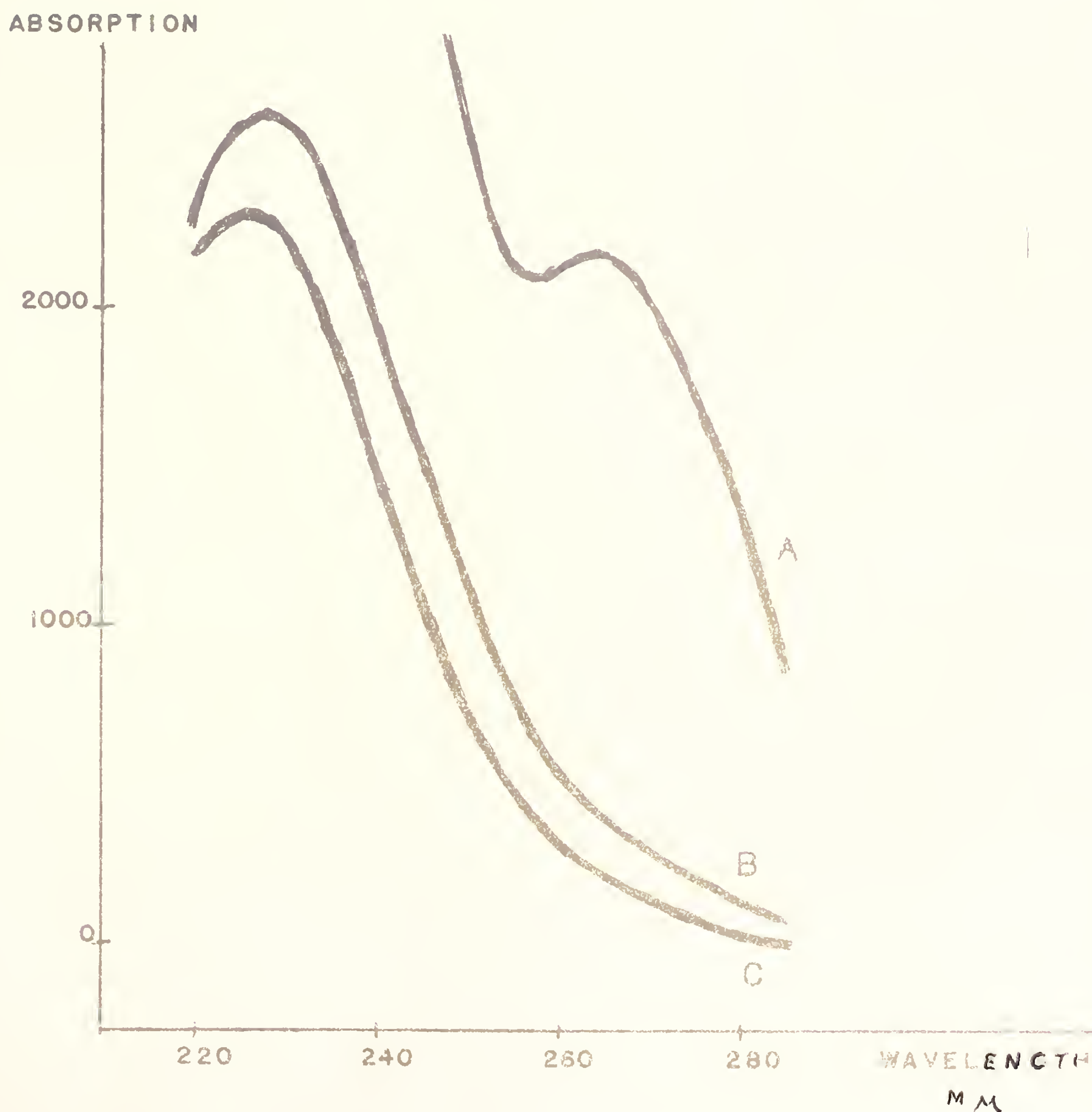
GRAPH I

2.18×10^{-4} M N,S DIBENZOYL CYSTEAMINE (A)

1.17×10^{-4} M N,N′ DIBENZOYL CYSTAMINE (B)

2.37×10^{-4} M N, n-BUTYL BENZAMIDE (C)

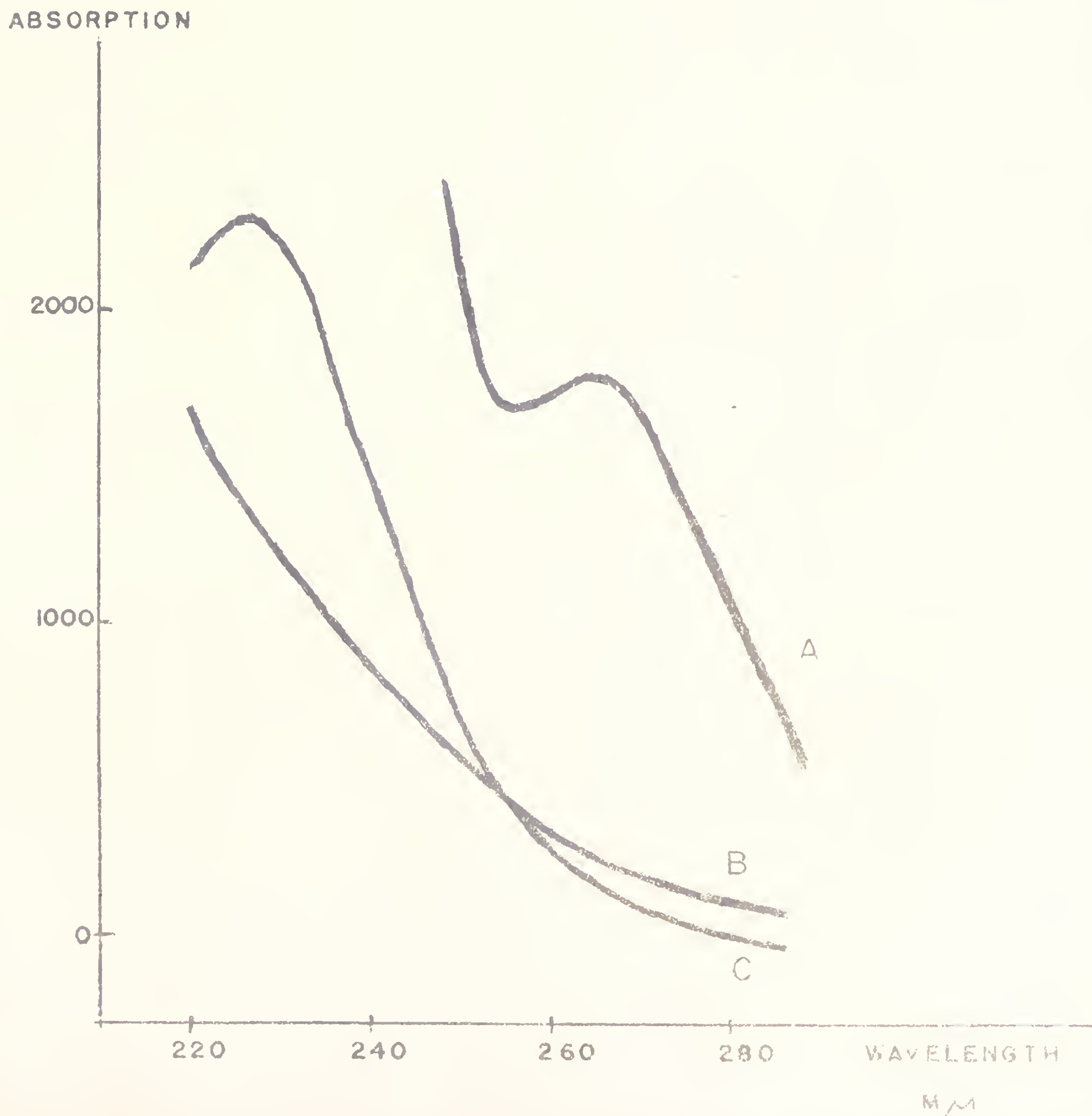
ABSORPTION SPECTRA

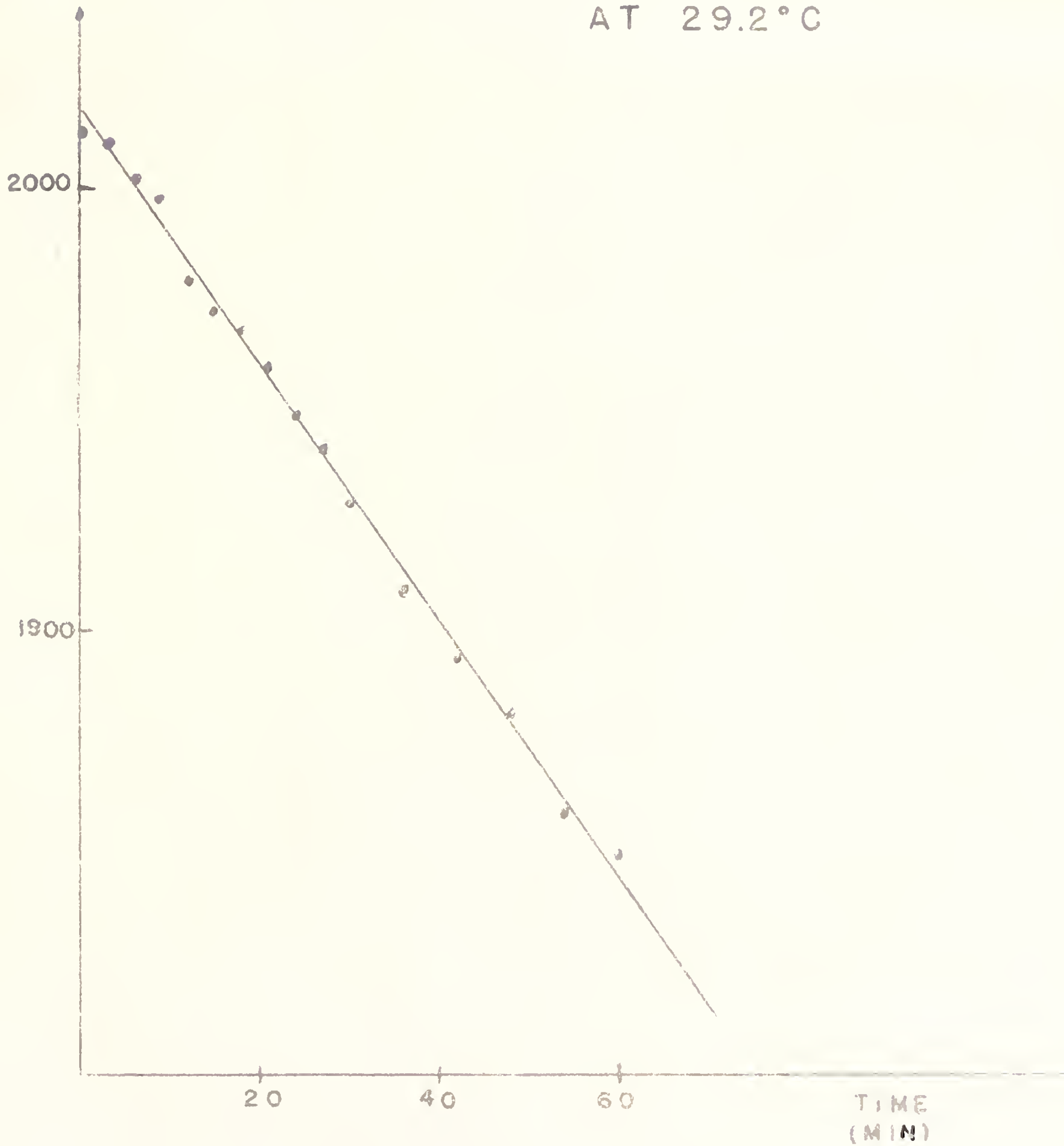


GRAPH 2

2.07×10^{-4} M N,S DIBENZOYL N-METHYL CYSTEAMINE (A)
1.05×10^{-4} M N,N' DIBENZOYL N,N' DIMETHYL CYSTAMINE (B)
2.37×10^{-4} M N. n-BUTYL BENZAMIDE (C)

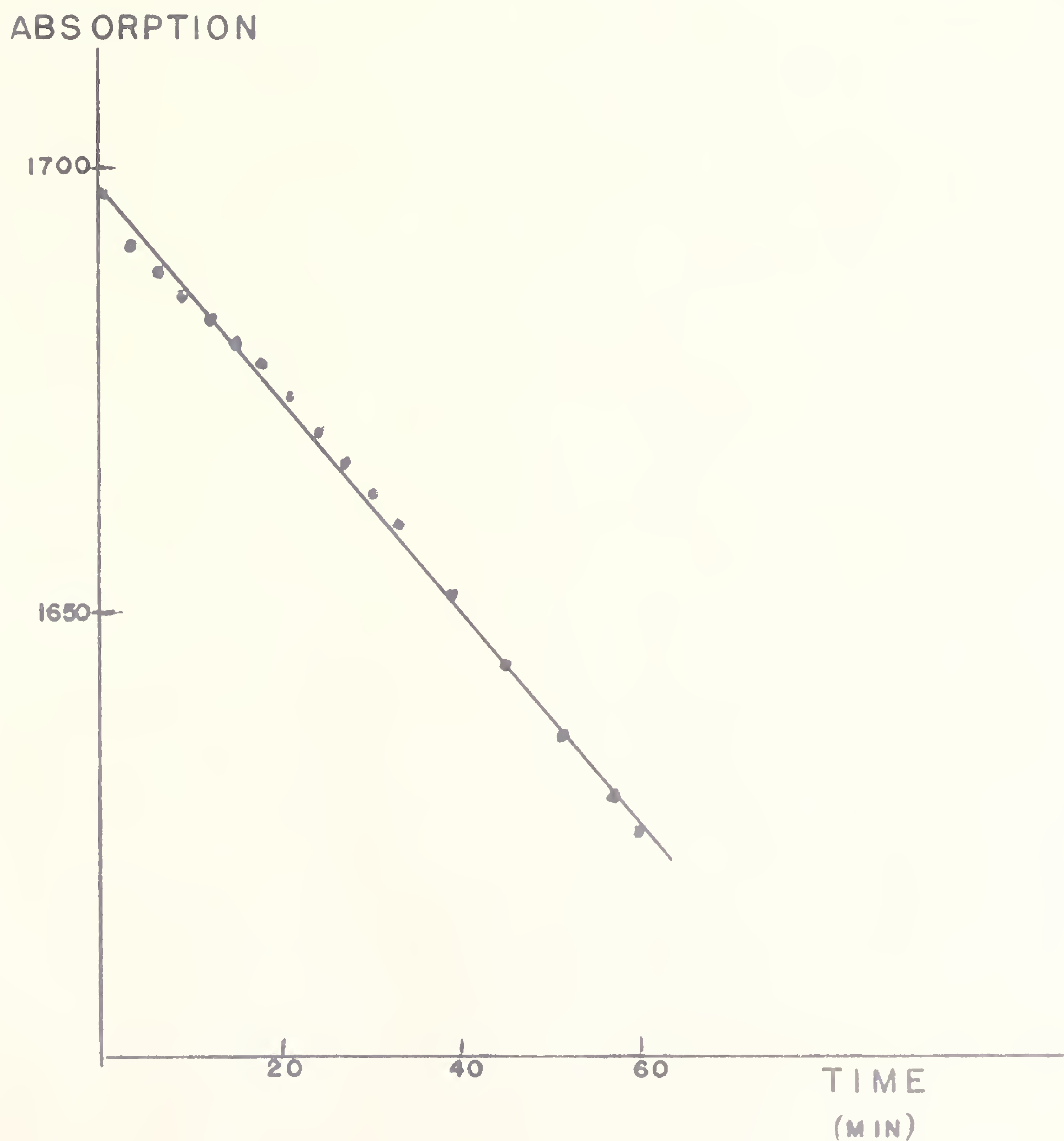
ABSORPTION SPECTRA




GRAPH 3
6.20 MG% N,S DIBENZOYL CYSTEAMINE
+.1 ML n-BUTYL AMINE
AT 29.2°C
ABSORPTION
2000
1900
20
40
60
TIME
(MIN)

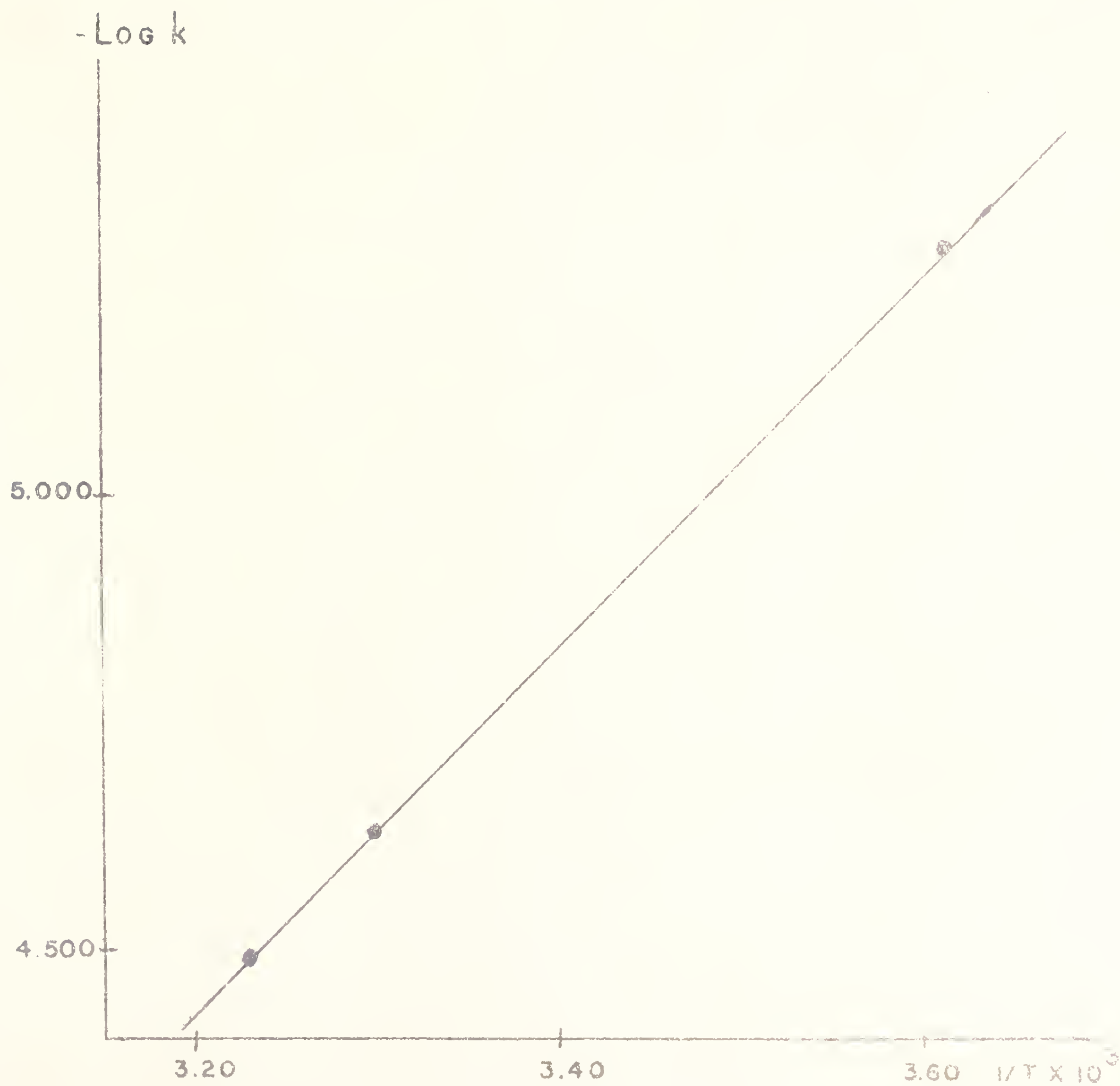
GRAPH 4

6.20MG% N,S DIBENZOYL N-METHYL
CYSTEAMINE
+ .1 ML BUTYL AMINE
AT 29.0°C



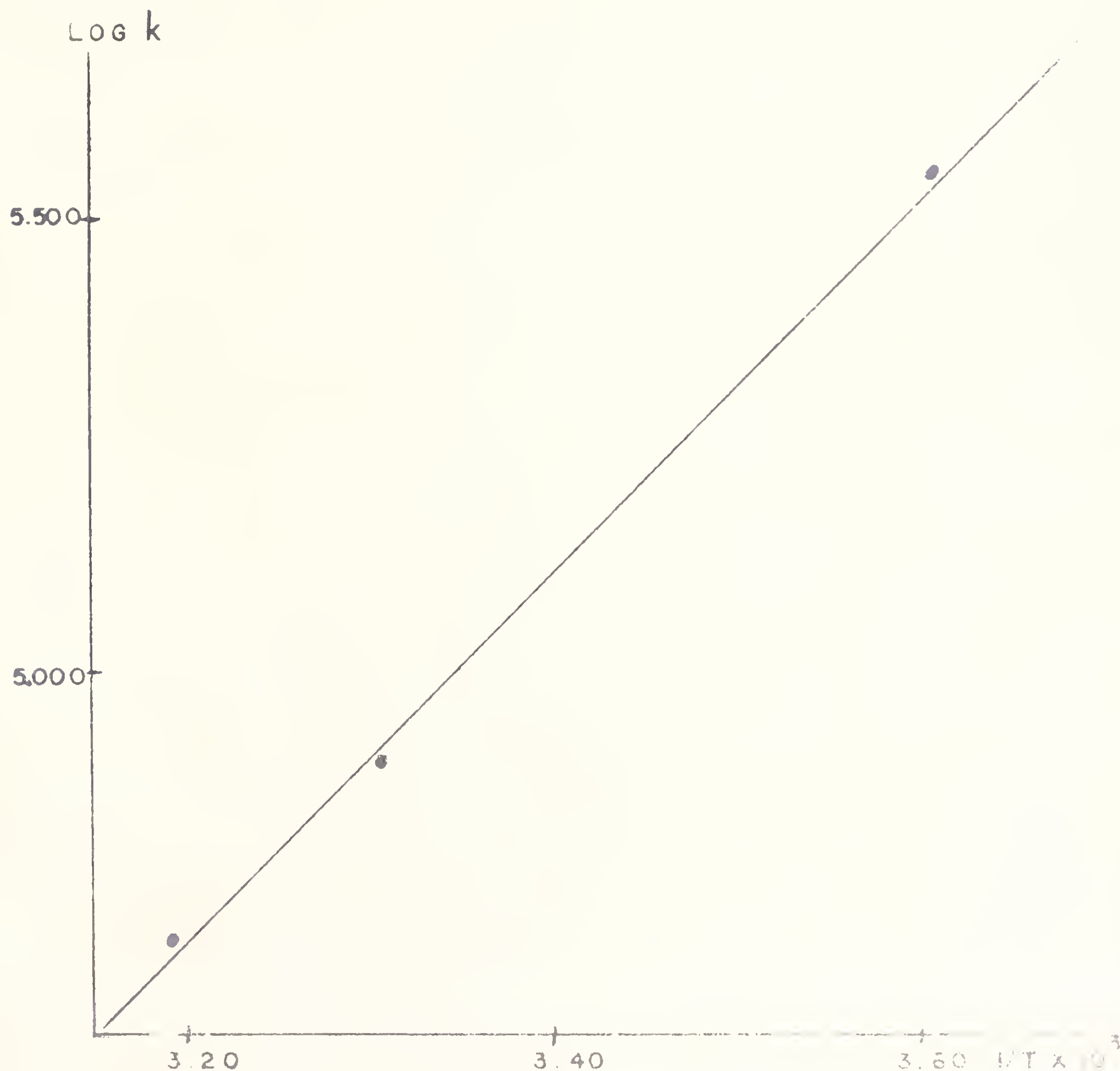
GRAPH 5

ENERGY OF ACTIVATION
FOR
N,S DIBENZOYL CYSTEAMINE
+ nBUTYL AMINE



GRAPH 6

ENERGY OF ACTIVATION
FOR
N,S DIBENZOYL N-METHYL CYSTEAMINE
+ n-BUTYL AMINE



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