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I. Polarographic determination of alpha-methyl-DL-cystine II. Synthesis of alpha substituted amino acids.

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I POLAROGRAPHIC DETERMINATION OF
α - METHYL - DL - CYSTINE

II SYNTHESIS OF ALPHA SUBSTITUTED
AMINO ACIDS

A THESIS

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ABSTRACT

PART I

Polarographic Determination of ∝- Methyl - DL - Cystine

A method of determining ∝- methyl - DL - cystine became necessary because of the synthesis of this alpha substituted amino acid. The polarographic determination of the substance in 0.1N hydrochloric acid, using thymol as a maximum suppressor, was investigated. The relationship of the diffusion current to the compound and to the temperature was studied. The influence of the thymol concentration and pH on the apparent half-wave potential was determined. A linear relationship of the diffusion current to the concentration of ∝- methyl - DL - cystine was observed in the range of 5×10^{-4} to 2×10^{-3} M. The system is not reversible.

PART II

Synthesis of Alpha Substituted Amino Acids

The chemical and physiological importance of alpha substituted cystine for further studies are discussed. A

literature survey was made of synthesized alpha substituted amino acids and methods of synthesis. Three different methods the Strecker, the Schmidt and the Curtius were used experimentally in the attempted synthesis of α-phenyl cystine.

ACKNOWLEDGMENTS

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PART I

POLAROGRAPHIC DETERMINATION OF α - METHYL - DL - CYSTINE

CHAPTER I

INTRODUCTION

The synthesis of ∝ - methyl - DL - cystine by Arnstein (1), which has been confirmed in this laboratory (6), made it desirable to establish a method of analysis for this compound.

Cystine has been determined colorimetrically, by Chinard and Hellerman (2), by reduction of cystine and using reagents which form colors in the presence of free sulfhydryl groups. Cystine can also be determined polarographically, and studies of this type have been reported by Kolthoff and Barnum (3).

Because ∝ - methyl - DL - cystine has a structure similar to cystine, it was thought feasible to attempt a polarographic estimation.

The oxidation potential of the biologically important cysteine-cystine system has been the subject of many investigations (4). However, the reduction of cystine at the dropping mercury electrode has received very little attention from investigators in the field, until the work of Kolthoff and Barnum (3) in 1941 and Kalousek, Grubner and Tochstein (9) in 1953.

Brdicka (5) found that when cystine was electrolyzed, in a buffer solution of 0.1N ammonium chloride and 0.1N ammonia, a polarographic wave was obtained, the height of which was observed to be proportional to the concentration of cystine. A somewhat more detailed study was made by Roncato (4).

He studied the polarographic reduction of cystine in unbuffered neutral, acid, and alkaline solutions of lithium chloride. However, his waves cannot be subjected to an exact analysis as his solutions were unbuffered (8) and the reduction potential depends on the pH;

$$RSSR + 2H^{+} + 2e \longrightarrow 2\ RSH$$

Kolthoff and Barnum found that cystine waves have peculiar characteristics and their interpretation is quite involved. They found that the addition of capillary-active substances such as phenol, thymol, gelatin or camphor suppressed the maximum. However, varying results were obtained and from a practical viewpoint thymol was found to be the most suitable for the elimination of the cystine maximum. Therefore, it was decided to adopt it for the determination of α - methyl - DL - cystine.

Kolthoff and Barnum found that the analysis of the current-voltage curves shows that the reduction of cystine at the dropping electrode does not occur reversibly and that the pH has a marked effect on the reduction potential. They also found that cystine may be determined polarographically at a pH of 1 using thymol as the maximum suppressor. The diffusion current was found to be proportional to the concentration.

CHAPTER II

EXPERIMENTAL

The ∝ - methyl - DL - cystine, prepared in this laboratory (6), was recrystallized three times from absolute ethanol prior to use in this study. A stock solution of 1×10^{-2} M ∝ - methyl - DL - cystine was prepared in 0.1N hydrochloric acid. A 1.2×10^{-3} M thymol solution in 0.1N hydrochloric acid was prepared for use as a maximum suppressor. The 0.1N hydrochloric acid was used as the supporting electrolyte as well as the solvent. The solutions used for analysis were prepared in 100 ml. volumetric flasks by adding the appropriate amounts of ∝ - methyl - DL - cystine and thymol from these stock solutions, and diluting to volume with 0.1N hydrochloric acid or buffer.

The solutions were transferred to a polarographic cell and nitrogen (purified by passing through ammoniacal cuprous chloride) was bubbled through for 5 minutes to remove the oxygen absorbed by aeration.

A Sargent (E. H. Sargent & Co.) Model XXI polarograph was employed for the study. A Heyrovský polarographic cell was employed throughout most of the work; an H-cell with saturated calomel electrode (S.C.E.) was used to determine the half-wave potential ($E_{\frac{1}{2}}$). Polarograms were run through the range of 0.0 to -1.0 volts. The drop rate was adjusted to 3 seconds and the temperature controlled to $\pm$ 0.1°C. Each polarographic determination was done in triplicate and the results reported as an average of these.

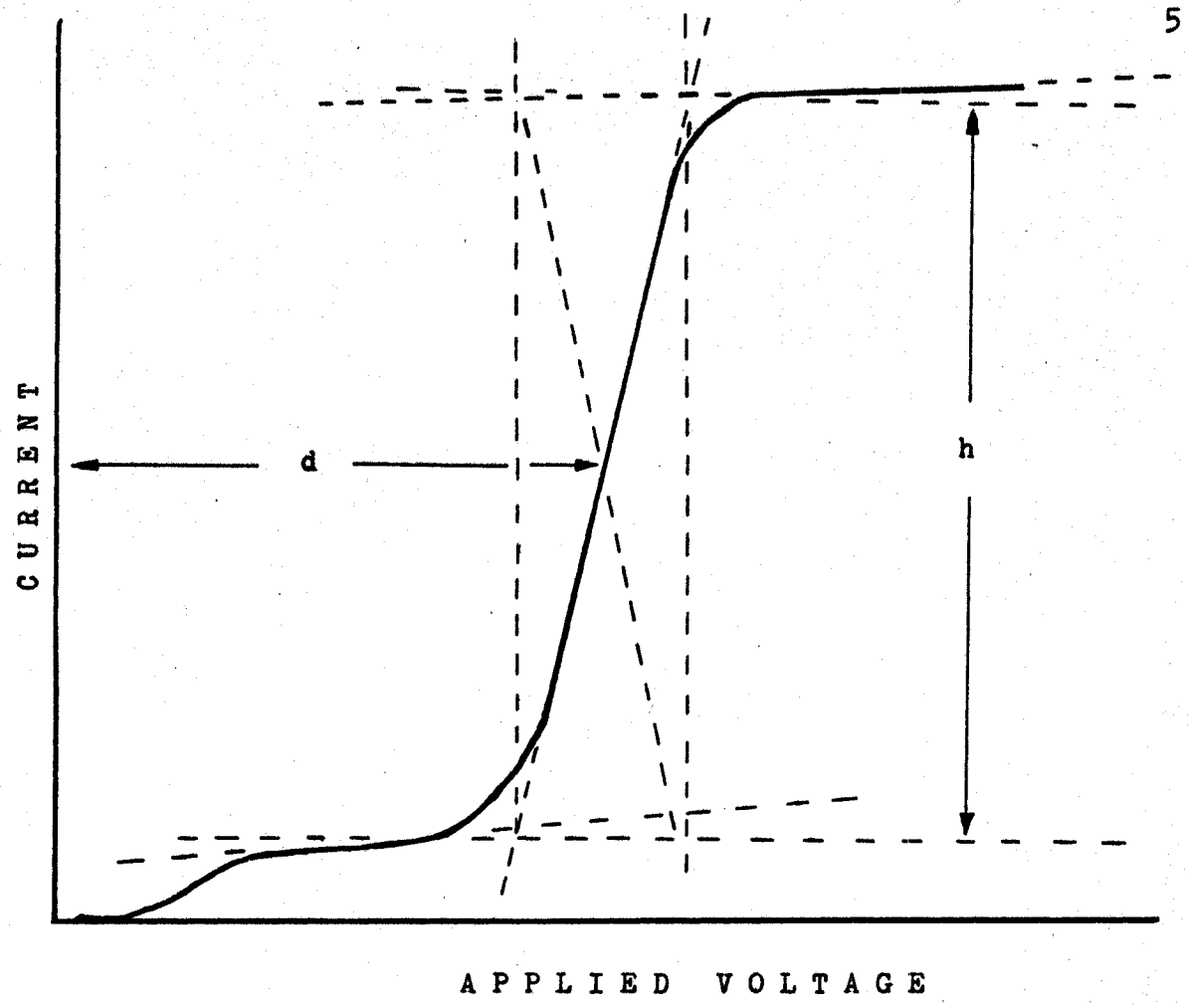


FIGURE 1

This is a typical example of a polarogram of ∝ - methyl - DL - cystine. The determination of the height of the diffusion curve and the distance for the half-wave potential are shown.

No damping was used in recording any of the polarograms obtained for this study.

The diffusion current was measured from the top of the first wave to the top of the second wave as there occurs a prewave with thymol (Figure 1). This type of prewave also occurs with cystine and has been studied by Kalousek, Grubner and Tochstein (9).

Except for the study of diffusion current dependence on temperature, where the height of the diffusion current represents the prewave plus the top wave, the diffusion currents and half-wave potentials are based on the top wave only wherever a prewave occurred.

The diffusion current was calculated by measuring the height, h, of the polarographic wave in millimeters (Figure 1) and substituting into the following relationship:

$$i_D = \text{Sens.} \times h$$

where i_D is the diffusion current and Sens. represents the sensitivity.

e.g. Sensitivity, Sens. = 0.020 μa per mm.
Height of wave, h = 169 mm.

Therefore: $i_D = 0.020 \times 169$

$= 3.38$ μa

All diffusion current values are reported to $\pm$ 0.005 μa.

The half-wave potentials were calculated by measuring the distance in inches from 0.0 volts to the middle of the diffusion curve, as shown in Figure 1.

This information was then substituted into the following:

$$E_{\frac{1}{2}} = (1/13.5) \times d$$

where 1/13.5 is a constant for the polarograph (a polarogram is 13.5 inches long for every span; since the span used is 1 volt, then 13.5 inches represents 1 volt on the polarogram, and the voltage at any time is 1/13.5 of the distance in inches from the beginning of the span) and d is the distance in inches from the beginning of the polarogram, which was 0.0 volt, to the half-wave potential.

e.g. Distance, $d = 7.27$ inches

Therefore $E_{\frac{1}{2}} = (-1/13.5) \times 7.27$

$= -0.538$ volts

All potential values are reported to $\pm$ 0.005 volt.

Half-wave Potential

The half-wave potential ($E_{\frac{1}{2}}$) measurements were determined for solutions containing 1×10^{-3} M α - methyl - DL - cystine with 1.2×10^{-4} M thymol, as the maximum suppressor, in 0.1N hydrochloric acid and 1×10^{-3} M α - methyl - DL - cystine with 4.8×10^{-4} thymol in 0.1N hydrochloric acid. The potentials were measured in an H-cell against a saturated calomel electrode (S.C.E.) with a Leeds and Northrup student potentiometer in order to substantiate the validity of the apparent applied potential of the polarograph.

The half-wave potentials were determined from the polarogram as shown in Figure 1 and following the calculations on pages 6 and 7. The potentials were observed to be -0.555 and -0.718 volt for thymol

concentrations of 1.2×10^{-4} and 4.8×10^{-4} M, respectively.

Capillary Constant

The Capillary Constant ($m^{2/3}\ t^{1/6}$) was calculated at 25°C and -0.555 volts.

Drop rate, t, was 3.00 seconds

Mass of one drop of mercury a) 0.00774 g
b) 0.00772 g
c) 0.00773 g

Average mass of mercury was 0.00773 g

Mass of mercury, m, per second in milligrams was

$$\frac{0.00773 \times 1000}{3} = 2.57 \text{ mg / sec}$$

Therefore $$m^{2/3}\ t^{1/6} = 2.57^{2/3} \times 3^{1/6}$$
$$= 2.253$$

Effect of Concentration on Diffusion Current

The effect of concentration of ∝ - methyl - DL - cystine on the diffusion current was studied at five different concentrations. Solutions of ∝ - methyl - DL - cystine (2×10^{-3}, 1.5×10^{-3}, 1×10^{-3}, 7.5×10^{-4}, and 5×10^{-4} M) were prepared with 1.2×10^{-4} M thymol as the maximum suppressor and 0.1N hydrochloric acid as the supporting electrolyte. Polarograms were obtained at 25°, 30° and 37.5°C, respectively.

The diffusion currents were determined (Table 1) by measuring the height of the polarographic wave, as shown in Figure 1, and following the calculations given on page 6.

TABLE I

EFFECT OF CONCENTRATION ON DIFFUSION CURRENT

Concentration α- Methyl - DL - Cystine, Mole/Liter	Diffusion Current, Microamperes[a]		
	25°C.	30°C.	37.5°C.
2.0×10^{-3}	8.66	8.89	8.03
1.5×10^{-3}	6.42	6.78	6.03
1.0×10^{-3}	4.19	4.60	3.98
7.5×10^{-4}	3.64	3.53	3.06
5.0×10^{-4}	2.28	2.36	2.01

[a] Average of three determinations.

Diffusion Current Dependence on Temperature

Polarograms of 1 x 10^{-3} M ∝ - methyl - DL - cystine using 1.2 x 10^{-4} M thymol were run at temperatures ranging from 0° to 45° C (Table II).

Effect of Thymol on Apparent Half-Wave Potential

Studies were made on the effect of thymol concentration on the shape of the polarographic wave. Solutions of 1 x 10^{-3} M ∝ - methyl - DL - cystine were analyzed at thymol concentrations of 2.5 x 10^{-5}, 1.2 x 10^{-4}, 2.4 x 10^{-4} and 4.8 x 10^{-4} M. The value of the apparent half-wave potential was calculated (Table IV). There was a change in the shape of the polarographic wave (Figure 2).

Effect of pH on Apparent Half-Wave Potential

Solutions of 1 x 10^{-3} M ∝ - methyl - DL - cystine were prepared using media of various pH levels according to Clark and Lubs (7) with 1.2 x 10^{-4} M thymol. Stock solutions of 0.2 M potassium chloride, 0.2 M hydrochloric acid, 0.2 M potassium acid phthalate, and 0.2 M NaOH, were prepared. The solutions for analysis were prepared in 100 ml. volumetric flasks by adding appropriate amounts from these solutions (Table III).

All pH measurements were made with a Beckman Model G pH meter. The solutions were analyzed at 21.1°C and the apparent half-wave potentials were determined (Table III). There was a distinct difference in the shape of the curves (Figure 3).

TABLE II

EFFECT OF TEMPERATURE ON DIFFUSION CURRENT

Temperature, °C.	Diffusion Current, μa.	
0.0	4.28	4.26
11.5	5.28	5.28
19.0	5.68	5.66
22.5	6.00	6.00
25.0	6.22	6.21
30.0	6.54	6.64
35.0	7.06	7.04
40.0	7.36	7.36
45.0	8.00	8.00

TABLE III.

EFFECT OF pH ON APPARENT HALF-WAVE POTENTIAL

pH[a]	Medium Used[b]	Apparent Half-Wave Potential, Volt	
0.12	IN HCl	-0.318	-0.320
1.10	0.2M KCl + 64.5 ml. 0.2N HCl[c]	-0.537	-0.540
1.97	0.2M KCl + 10.6 ml. 0.2N HCl[c]	-0.597	-0.575
3.00	0.2M Potassium acid phthalate + 20.3 ml. 0.2N HCl[c]	-0.663	-0.666
3.48	0.2M Potassium acid phthalate + 6.0 ml. of 0.2N HCl[c]	-0.727	-0.726
3.96	0.2M Potassium acid phthalate + 0.40 ml. 0.2N NaOH[c]	-0.810	-0.800

[a] Values represent final solution to be analyzed.

[b] Buffers were prepared according to Clark and Lubs (7).

[c] Amount of acid or base required to prepare 200 ml. of buffer solution.

TABLE IV

EFFECT OF THYMOL CONCENTRATION ON APPARENT HALF-WAVE POTENTIAL

Thymol Concentration Mole/Liter	Apparent $E_{\frac{1}{2}}$, Volt		Temperature $^{o}C.$
2.4×10^{-5}	-0.343	-0.343	21.7
1.2×10^{-4}	-0.555	-0.555	21.9
2.4×10^{-4}	-0.652	-0.652	21.9
4.8×10^{-4}	-0.723	-0.723	18.2

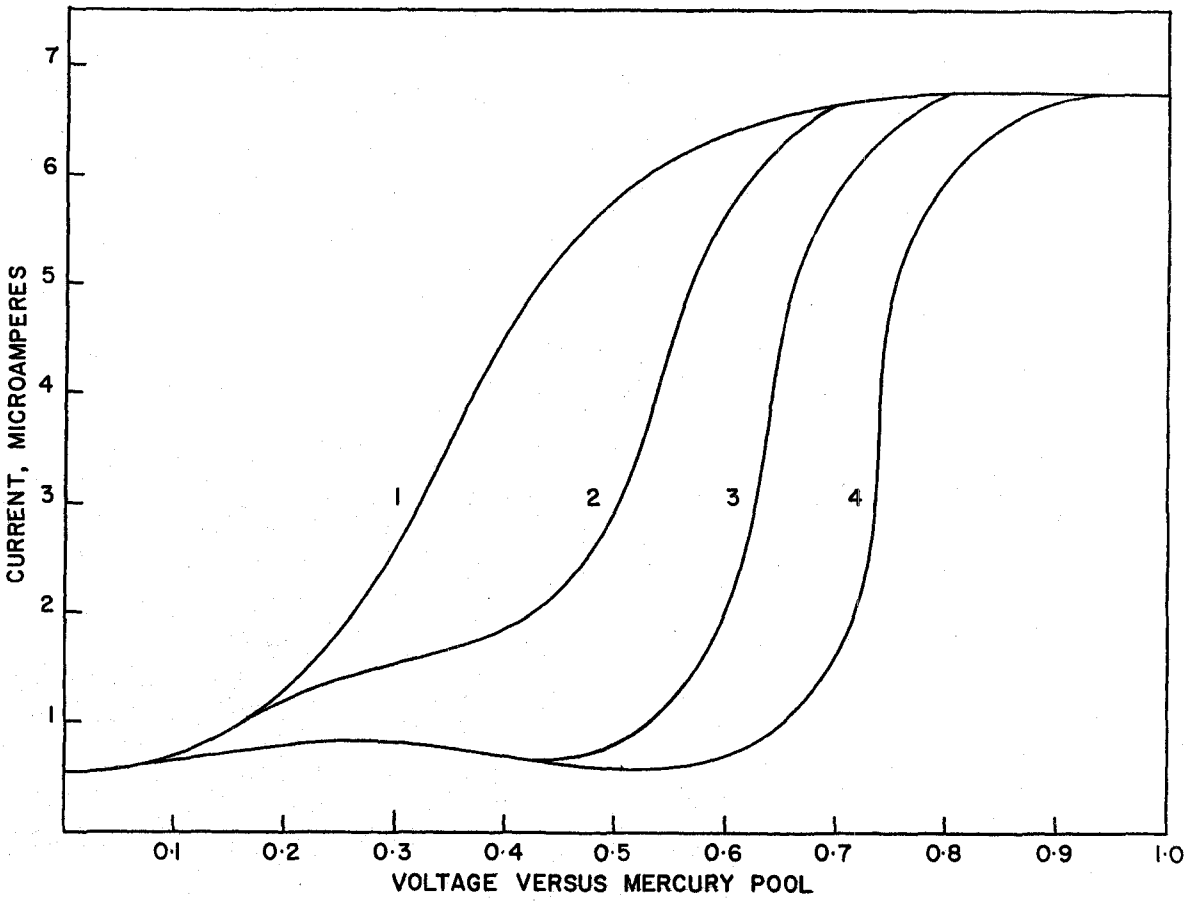


FIGURE 2

Effect of thymol concentration on shape of polarogram

1. 2.4×10^{-5}M
2. 1.2×10^{-4}M
3. 2.4×10^{-4}M
4. 4.8×10^{-4}M

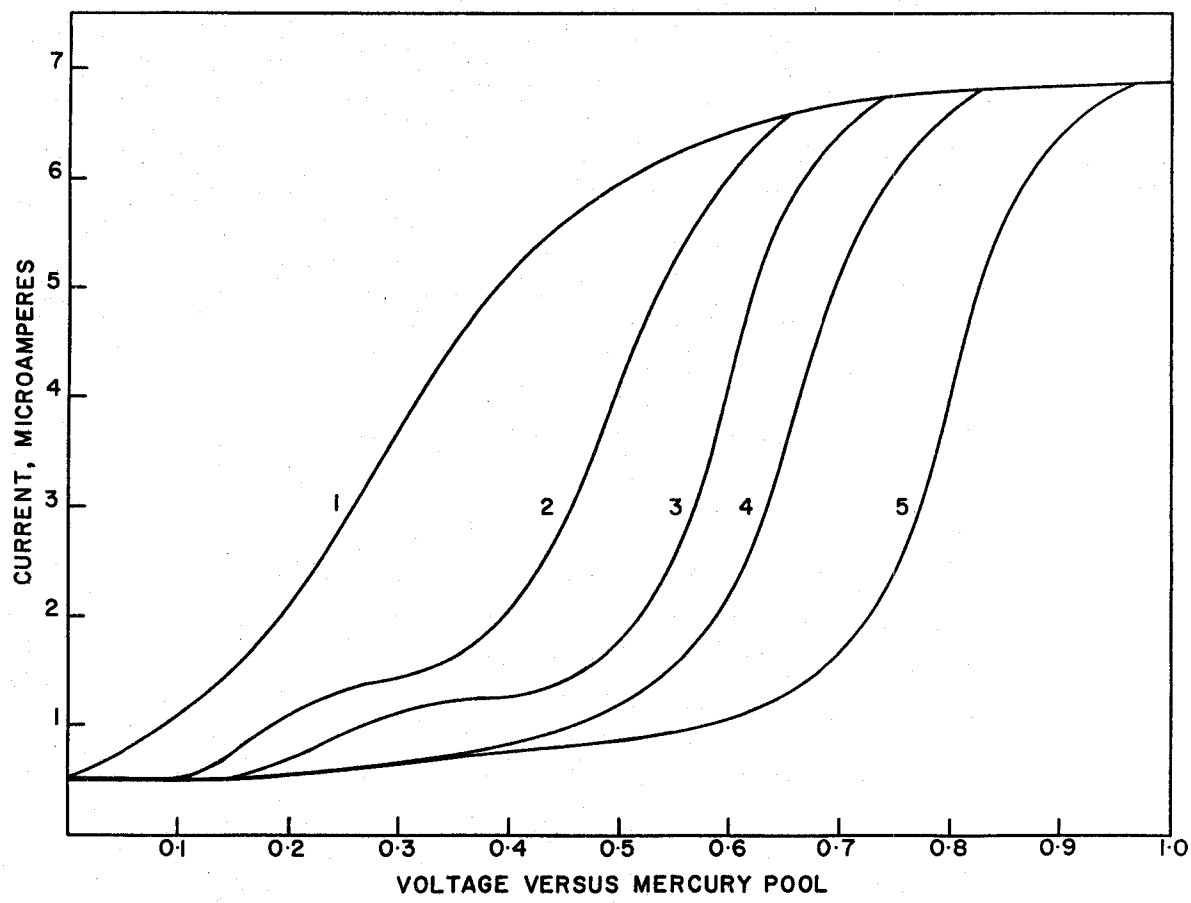


FIGURE 3

Effect of pH on apparent half-wave potential
(See Table III for buffers)

1. pH 0.12
2. pH 1.10
3. pH 1.97
4. pH 3.00
5. pH 3.96

Effect of Digestion on Polarographic Wave of ∝ - Methyl - DL - Cystine.

In order to ascertain that no substance in the reagents was responsible for the wave obtained with ∝ - methyl - DL - cystine duplicate samples of 1 x 10^{-2} M ∝ - methyl - DL - cystine were digested according to the method of Koch and McMeekin (10).

5 ml. of 1 x 10^{-2} M ∝ - methyl - DL - cystine were transferred to a pyrex test tube, and 1 ml. of 1:1 sulfuric acid was added along with a quartz pebble. This was heated over a microburner to evaporate the water. When charring began and white fumes appeared in the tube, the size of the flame was reduced. Heating was continued until no further blackening occurred. The tube was cooled for approximately one minute, and then 1 drop of 30 per cent hydrogen peroxide was added. The tube was heated to boiling. The above procedure was repeated until the solution was decolorized. Finally the mixture was boiled gently for 5 minutes. It was cooled and transferred to a 50 ml. volumetric flask and diluted to the mark with 0.1N hydrochloric acid and thymol. This procedure was done in duplicate along with two distilled water blanks.

The polarographic analysis of the digests in 0.1N hydrochloric acids at 19.4° C. using 1.2 x 10^{-4} M thymol revealed that the characteristic wave was absent in both the blanks and the samples.

Diffusion Coefficient and Reversibility of the Reaction.

The diffusion coefficient for a 1 x 10^{-3} M solution of ∝ - methyl - DL - cystine at 25°C., using

1.2×10^{-4} M thymol, was found by substituting in the Ilkovič equation.

$$i_D = 607\ n\ D^{\frac{1}{2}}\ C\ m^{2/3}\ t^{1/6}$$

Where Capillary constant, $m^{2/3}\ t^{1/6} = 2.253$

Diffusion current, $i_D = 4.19$ μa

Concentration in millimoles, $C = 1$

Number of electrons, $n = 2$

Diffusion coefficient $D = ?$

Substituting:

$$4.19 = 607 \times 2 \times D^{\frac{1}{2}} \times 1 \times 2.253$$

$$D = 2.35 \times 10^{-6}\ cm.^2 \text{ per second}$$

The reversibility of the reaction was tested by plotting E vs. $\log i_D - i / i^2$ for 1×10^{-3} M ∝- methyl - DL - cystine at 20°C. for thymol concentrations of 1.2×10^{-4} and 4.8×10^{-4} M, respectively. The potential (E) was measured vs. S.C.E. at various points on the polarograms and the diffusion currents calculated (Table V).

Straight lines were obtained for both thymol concentrations with slopes of 0.0817 and 0.0533 (Figure 4) for thymol concentrations of 1.2×10^{-4} and 4.8×10^{-4} M, respectively.

The half-wave potential ($E_{\frac{1}{2}}$) was observed to be -0.555 and -0.718 volt for thymol concentrations of 1.2×10^{-4} and 4.8×10^{-4} M, respectively.

TABLE V.

VALUES OF E AND $i_D - i / i^2$

Thymol Concentration 1.2×10^{-4} M		Thymol Concentration 4.8×10^{-4} M	
E^a, Volt	$\frac{i_D - i}{i^2}$	E^a, Volt	$\frac{i_D - i}{i^2}$
-0.41	28.42	-0.62	26.00
-0.45	4.08	-0.65	7.55
-0.50	1.43	-0.67	3.30
-0.55	0.476	-0.72	0.35
-0.60	0.102	-0.75	0.06

[a] The potential (E) was determined in an H-cell vs. S.C.E. at 20°C.

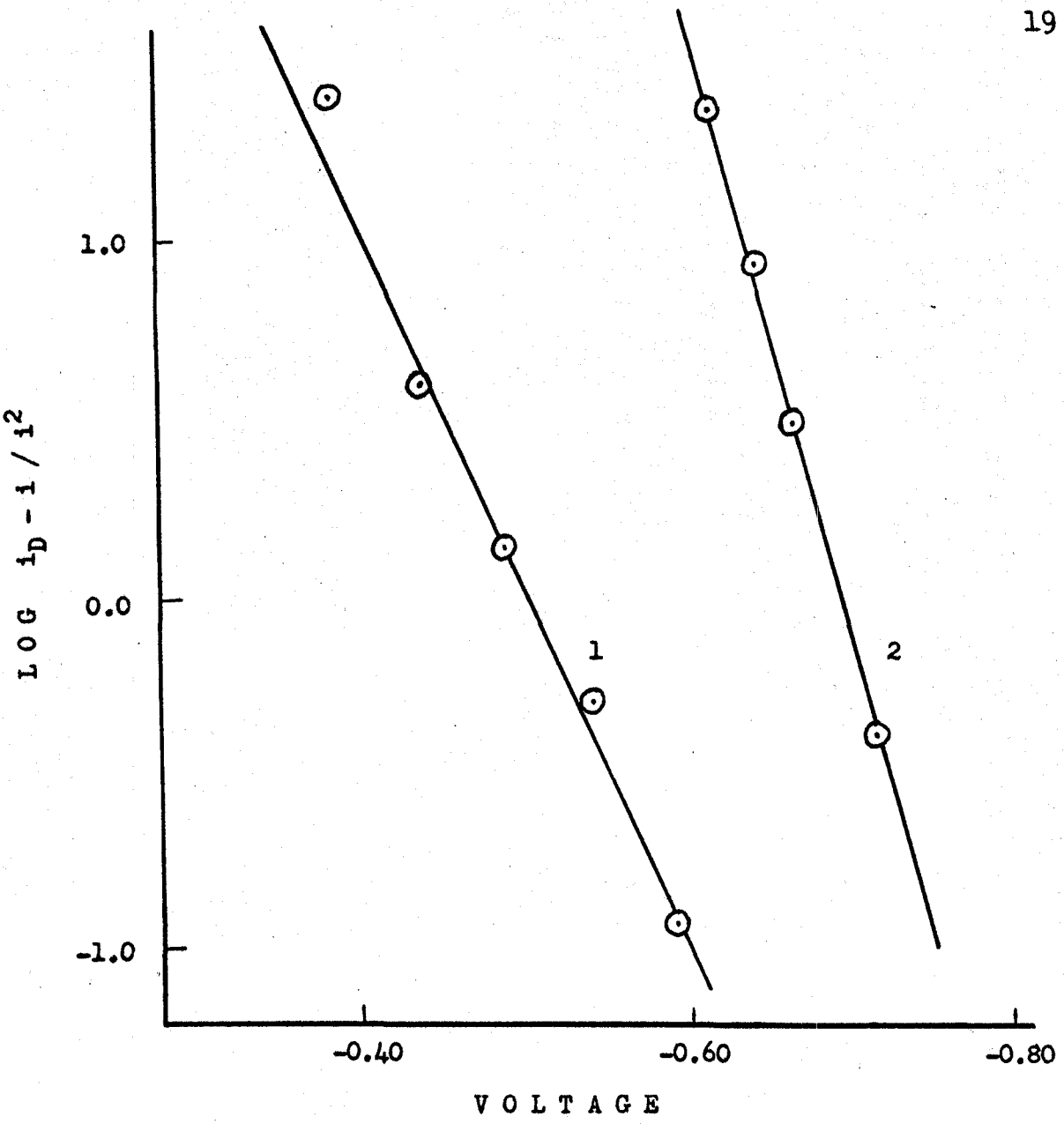


FIGURE 4

Voltage is plotted against log $i_D - i / i^2$. Curve (1) represents 1.2 x 10^{-4} M thymol in 1 x 10^{-3} M ∝ - methyl - DL - cystine; (2) represents 4.8 x 10^{-4} M thymol in 1 x 10^{-3} M ∝ - methyl - DL - cystine.

CHAPTER III

SUMMARY

The effect of concentration of ∝- methyl - DL - cystine, using 1.2×10^{-4} M thymol as the maximum suppressor and 0.1N hydrochloric acid as solvent and supporting electrolyte on the diffusion current, was found to result in a linear relationship (Table I). A linear relationship was also observed between temperature and total diffusion current (Table II).

The higher the pH, the more nearly S-shaped the polarograms became (Figure 3). The polarograms had different shapes (Figure 3) and the apparent half-wave potential was observed to decrease with increase in pH. Polarographic analysis of the digests revealed that the characteristic wave was absent in both the blank and the samples of ∝- methyl - DL - cystine. This was taken as evidence that no substance present in the reagents was responsible for the wave normally obtained with ∝- methyl - DL - cystine.

The value of the apparent half-wave potential changes with thymol concentration (Table IV) because of a change in the shape of the polarographic wave (Figure 2). A well defined S-shaped curve occurred at a thymol concentration of 4.8×10^{-4} M. By substitution in the Ilkovič equation, the diffusion coefficient for 1×10^{-3} M ∝- methyl - DL - cystine at 25°C., using 1.2×10^{-4} M thymol, was found to be 2.35×10^{-6} $cm.^2$ per second; the diffusion current was 4.19 μa. and the capillary constant

2.253. The value of the diffusion coefficient obtained is of the same order as that reported by Kolthoff and Barnum (3) for cystine (5.3×10^{-6} $cm.^2$ per second). The value for the substituted cystine is less because of the greater bulk effect of the molecule.

The reversibility of the reaction was tested by plotting E vs. log $i_D - i / i^2$ for 1×10^{-3} M α- methyl - DL - cystine at thymol concentrations of 1.2×10^{-4} and 4.8×10^{-4} M which revealed straight lines with slopes of 0.0817 and 0.0533, respectively (Figure IV). These values differ from the theoretical slope (0.0295). In this respect the results are similar to those of Kolthoff and Barnum (3) and Kalousek, Grubner and Tochstein (9), who concluded that the cystine reduction is not reversible.

CONCLUSION

The analyses of the current-voltage curves show that the reduction of ∝- methyl - DL - cystine at the dropping mercury electrode does not occur reversibly and that the reduction potential was markedly affected by pH and by thymol concentration. The diffusion current was found to be proportional to the concentration of ∝- methyl - DL - cystine as well as to temperature. Solutions of ∝- methyl - DL - cystine may be determined polarographically within the concentration range of 2×10^{-3} to 5×10^{-4} M using 1.2×10^{-4} M thymol as the maximum suppressor and 0.1N hydrochloric acid as solvent and supporting electrolyte.

PART II

SYNTHESIS OF ALPHA SUBSTITUTED AMINO ACIDS

CHAPTER I

INTRODUCTION

From the comparison of the polarographic results of cystine by Kolthoff and Lingane (3) and of ∝-methyl - DL - cystine in this laboratory (11) it was felt that it would be of interest to study the effect of other substituent groups in the alpha position of cystine. The object of the study is to investigate the steric effect on the half-wave potential, the diffusion current, and the shape of the polarograms.

In order to carry out this investigation the synthesis of the following compounds is necessary; ∝-ethyl cystine, ∝-propyl cystine, ∝-isobutyl cystine, ∝-isopropyl cystine, ∝-2° butyl cystine, ∝-phenyl cystine, ∝-naphthyl cystine, and ∝- tertiary butyl cystine. It is to be noted that the above compounds represent different degrees of steric effect which should then be manifested by a change in the half-wave potential, the diffusion current, and the shape of the polarograms.

Cystine and cysteine constitute a typical oxidation-reduction system, each half of the system differing from the other by two electrons and two hydrogen atoms (12);

$$2 \text{ cysteine} \rightleftharpoons \text{Cystine} + 2H^{+} + 2e$$

The oxidation of cysteine can be effected by atmospheric oxygen:

$$2R\text{-}SH \xrightarrow{(O_2)} R\text{-}S\text{-}S\text{-}R$$

However, this is a slow process and it can be enhanced by such metals as iron (13), nickel and manganese (14). Cystine is usually reduced to cysteine by the action of tin metal on hydrochloric acid solutions of cystine (15) or by hydrogen with a catalyst.

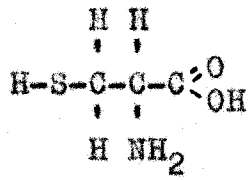
The presence of both acidic and basic groups in the amino acid molecule leads to partial internal neutralization with the formation of a dipolar ion (16):

$$HS\text{-}CH_2\text{-}\underset{\displaystyle NH_2}{\overset{\displaystyle R}{\underset{|}{\overset{|}{C}}}}\text{-}COOH \rightleftharpoons HS\text{-}CH_2\text{-}\underset{\displaystyle NH_3^+}{\overset{\displaystyle R}{\underset{|}{\overset{|}{C}}}}\text{-}COO^-$$

Zwitterion

The dipolar ion produces a saltlike character and gives amino acids a high melting point. Many decompose before they melt. α- Amino acids, like α- hydroxy acids, form cyclic derivatives. The formation of these cyclic diamides interferes with a simple synthesis of polyamides (proteins) (17).

Cysteine has three functional groups: the sulfhydryl group (-SH), the carboxyl group (-COOH) and the amine group ($-NH_2$) as shown by the following structure:



Structure of Cysteine

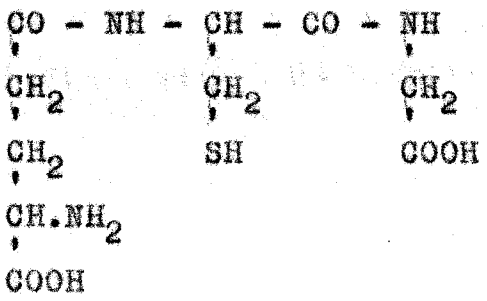
Thus if substitution could be effected in the alpha position it should have an influence on the reactivity of these functional groups.

Amino acids readily form chelates with copper salts if a primary or secondary amino group is close to the carboxyl group (18). In general, cysteine has the basic requirements of a chelating agent with the acid group (-COOH) and the basic amine group ($-NH_2$) both situated so as to form a six-membered, strain-free ring.

Amino mercaptans also react with acqueous solutions of heavy metal salts to form highly insoluble mercaptides. Kolthoff and Sticks (19) obtained the copper mercaptide of cystine, RSCu, by reacting cystine with cuprous chloride in near neutral solution. Lead, zinc and mercury also readily form mercaptides (20).

Substituent groups affect the acid base strength as well as the solubility of these complex ions. The addition of hydrocarbon substituents generally decreases the solubility of the precipitate and so increases the effectiveness of the agent (21). It would be of interest to study the influence of various hydrocarbon groups in the alpha position of cystine on the solubility of their chelates. It would also be interesting to study the effect of these substituent groups on the chelating strength of the substituted cysteines.

In biochemistry, cysteine and glutathione are often referred to together because they undergo similar reactions and have similar physiological effects. Glutathione is a tripeptide which is biosynthesized from the component amino acids L-glutamic acid, L-cysteine and glycine (22):



Structure of Reduced Glutathione (23).

Cysteine and glutathione form an oxidation-reduction equilibrium which is common with thiol-disulfide systems (24):

$$2GSH + CSSC \rightleftharpoons GSSG + 2CSH$$

Rapkine (25) and Dickens (26) found that cysteine and glutathione combine with halogen acids giving the thio ethers of cysteine and glutathione. According to Rapkine, the reaction with cysteine is faster than with glutathione. Furthermore, the rate of reaction with cysteine was dependent on the pH value of the solution. Not all halogen acids react with thiols; cysteine was found to react with iodoacetate, bromoacetate, and chloro-acetate, but not with fluoroacetate:

$$R\text{-}SH + Hal\text{-}CH_2\text{-}COO^- \longrightarrow RSCH_2\text{-}COO^- + H\text{-}Hal$$

The rates were those of bimolecular reactions. It would be of interest to see if substituent groups in the alpha position of cysteine would have any effect on these reaction rates. At least the steric effect of these groups on the rate of saponification of the ethyl esters of the alpha substituted cystine should be significant.

most of the sulfur proteins in the body are represented by the methionine and cystine present, though small amounts of cysteine can be found. Cystine is produced in the metabolic breakdown of methionine, which is an essential amino acid (27). Therefore, it is possible to replace cystine with methionine; however, the presence of cystine in the diet decreases the demand for the formation of cystine from methionine.

Cysteine participates in a novel form of detoxication in the formation of mercapturic acids (28). Aromatic hydrocarbons and many of their monohalogenated derivatives are conjugated with cysteine in the body, the cysteine moiety is then acetylated, and the resulting mercapturic acid excreted in the urine. These include benzene, polycyclic hydrocarbons (e.g. naphthalene), and ring halogenated hydrocarbons (e.g. bromobenzene).

The sulfhydryl and disulfide groups present in amino acids, peptides, proteins, and enzymes are known to play important biological and physiological roles (29). Cysteine has been found to be in intact protein molecules, but is only obtained in the products of protein hydrolysis if precautions are taken against oxidation, since the (-SH) of cysteine is readily converted to the (- S - S -) of cystine.

The sulfhydryl groups of protein have been found to be essential in the reactivity of a great number of enzymes and as binding sites between protein and some prosthetic groups. Many enzymes contain sulfhydryl groups derived from the side-chains of cysteine residues. Blockage through chemical transformation of these groups results in inhibition of the enzymes (30). An example is the conversion of sulfhydryl groups to disulfides with oxidants such as ferricyanide:

$$2\ R\text{-}SH \xrightarrow[-2H]{\text{Ferricyanide}} R - S - S - R$$

Enzymes catalyze the combination of the protein moiety with the substrate to form the "Michaelis-Menten enzyme - substrate complex". This complex then breaks down to yield the enzyme plus the product. For example, D - glyceraldehyde - 3 - phosphate dehydrogenase converts 3 - phospho - glyceraldehyde to 1 - 3 - diphosphoglyceric acid:

$$\text{Enzyme + Substrate} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES^{*} \xrightarrow{k_3} \text{Enzyme + product.}$$

* "Michaelis-Menten Complex"

The formation and dissociation of the Michaelis-Menten Complex, as well as the equilibrium constants (k_1, k_2 and k_3) are influenced by the side chains of the protein molecule which contain sulfhydryl, amine, amide, carboxyl, and phenol groups. Therefore, it would be conceivable to think that a substituent group in the alpha position to one of these groups would have some influence as well.

It has been found that sulfhydryl content increases steadily with cell growth and reaches a maximum just before cell division. Upon division the sulfhydryl content at first is low and increases again until the second division occurs. To illustrate this, Rapkine (31) added small amounts of mercuric chloride to fertilized sea urchin eggs until mitosis was arrested completely. Mitosis was again initiated when cysteine was added.

It has also been found that there is a greater amount of thiol present in tumorous tissue than in non-tumorous tissue. The mechanism of thiols in the growth and division of cells is unknown. If, however, an unreactive sulfhydryl protein could be substituted for a reactive one then the rapid growth and division of cells in a tumor could be arrested. That is, if an unreactive amino acid, such as an alpha substituted cysteine, could be substituted for a reactive amino acid and inhibit the enzyme, the reaction will only go as far as the Michaelis-Menten Complex and would not break down into the desired product, thus slowing down the overall process. In other words it would act as a metabolic antagonist:

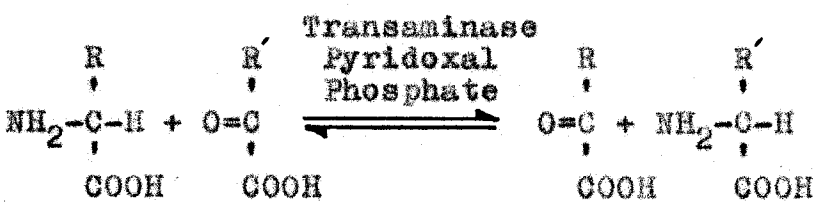
$$\text{Enzyme} + \text{Substrate} \rightleftharpoons \underset{\text{(unreactive)}}{ES^{*}} \longrightarrow \text{No product}$$

The effect of ionizing radiations upon the sulfhydryl and disulfide groups is of primary concern in radiation biochemistry, and has been recently reviewed by Barron (32). Cysteine and cystine have long been the object of radiochemical investigations because the

information from such studies is of help in understanding the radiation chemistry of more complex sulfhydryl and disulfide compounds.

Ionizing radiation oxidizes the sulfhydryl group (-SH) to the disulfide (-S-S-). Thus radiation inhibits sulfhydryl enzymes and lowers free sulfhydryl groups essential in cell growth. Sulfhydryl compounds such as glutathione and cysteine are more susceptible to oxidation by radiation than most sulfhydryl enzymes. Patt et al. (33) and Chapman et al. (34) found it possible experimentally to protect rats against lethal doses of radiation after injecting them with cysteine or glutathione. Therefore, derivatives of cysteine may be a step closer to the problem of radiation sickness.

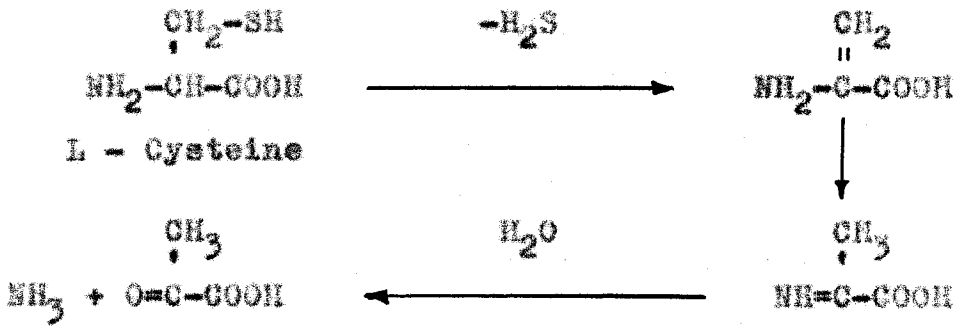
The metabolism of the amino acids involves deamination and formation of the corresponding keto acids preliminary to oxidation and the production of energy. The amino group of certain amino acids are transferred to the keto acid corresponding to the amino acid, thereby effecting amino acid - keto acid interconversion (35). This process is called transamination. The overall reaction may be represented by:



However, it was found by Umbreit (36) that α-methylglutamic acid inhibits decarboxylation, glutamine breakdown and glutamotransferase, and is inert to transamination and dehydrogenation. Alpha methyl substituted aliphatic amino acids (such as alanine, valine and serine)

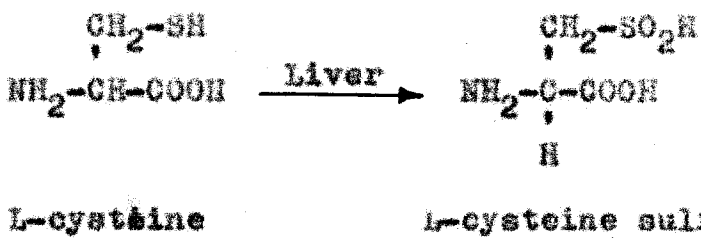
were inert to metabolic reactions. Thus, it is felt that alpha substituted cysteines would also be worth studying as metabolic antagonists.

Various flavoproteins usually catalyze the oxidative deamination of amino acids. However, deamination of some amino acids is brought about by means of a non-oxidative reaction with enzymes. Deamination of cysteine can be effected by cysteine desulfhydrase which catalyzes the removal of the elements of hydrogen sulfide from cysteine (37):



It can be seen from the above mechanism that if the alpha hydrogen were substituted that, unless this substituent group was removed by cysteine desulfhydrase, the reaction would be inhibited and the substitute cysteine would act as an anti-metabolite.

L-Cysteine is converted to L-cysteine sulfinic acid in the liver which then is further metabolized (38):



Here again it would be interesting to see if steric effect has any influence on the reaction.

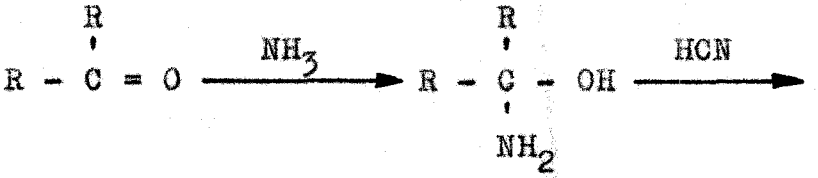
Therefore, it can be seen that the synthesis of alpha substituted cystines could lead to further studies which are of importance in the metabolism of cysteine and cystine.

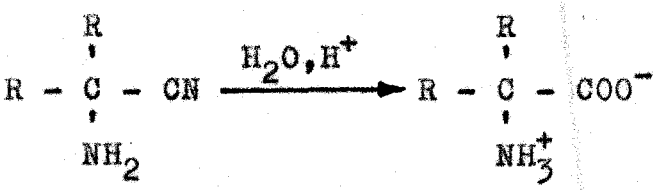
CHAPTER II

LITERATURE SURVEY OF ∝ - AMINO ACIDS SYNTHESIZED AND METHODS OF SYNTHESIS

The methods of alpha substituted amino acid synthesis reported in the literature are, for the most part, modified methods based on the Strecker, Hofmann, Schmidt, and Curtius reactions.

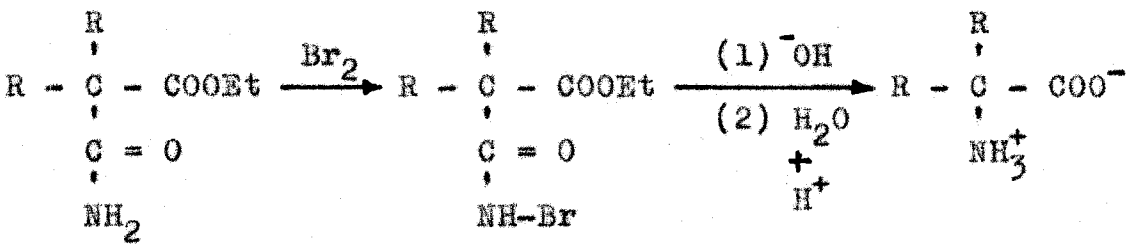
The Strecker synthesis was employed by Arnstein (1) in the synthesis of ∝- methyl - DL - cystine. This reaction consists of the reaction of a ketone with a mixture of ammonium chloride and sodium cyanide followed by acid hydrolysis of the resulting amino nitrile. The first stage of the reaction involves the formation of ammonium cyanide which dissociates into ammonia and hydrogen cyanide. Reaction of ammonia with the ketone gives the ammonia addition product, which reacts with hydrogen cyanide to give the amino nitrile:





Hydrolysis with dilute acid of the amino nitrile gives the amino acid.

The Hofmann reaction was employed by Laing Li (38) in the synthesis of alkyl amino acetic acids. In this reaction, an amide is converted to an amine of one less carbon atom by treatment with bromine (or chlorine) and alkali. In effect the carbonyl group of the amide is eliminated;



The reaction proceeds in two stages. The first stage consists in the formation of a bromoamide by the substitution of bromine for one of the hydrogen atoms attached to nitrogen and in a position activated by the adjacent carbonyl group. In the second stage, the bromoamide when warmed with excess alkali rearranges to an isocyanate which is hydrolysed in the reaction medium to the carbamic acid. The carbamic acid decomposes readily to yield carbon dioxide and the desired amine.

The Curtius reaction was used extensively by Gagnon et al. (39) in their synthesis of α- amino acids. This reaction is quite similar to the Hofmann reaction although it does not suffer the same limitations. The reaction is carried out on acid hydrazides which are prepared by warming acid esters with a solution of hydrazine. The hydrazides are converted into acid azides on treatment with nitrous acid. Azides on warming, decompose into isocyanates as in the Hofmann reaction:

$$R-\overset{O}{\overset{\|}{C}}-OC_2H_5 \xrightarrow{NH_2-NH_2} R-\overset{O}{\overset{\|}{C}}-NH-NH_2 \xrightarrow[HCl]{HONO} R-\overset{O}{\overset{\|}{C}}-N_3$$

However, the reaction is usually carried out by warming the azide in alcohol producing the urethane, which on hydrolysis yields the primary amine;

$$R-\overset{O}{\overset{\|}{C}}-N_3 \xrightarrow{EtOH} R-\underset{H}{N}-COOEt \xrightarrow{H^+,\ H_2O} R-NH_2 + CO_2 + EtOH$$

The Schmidt reaction involves the reaction of hydrazoic acid with a carbonyl compound in the presence of concentrated sulfuric acid or certain other acids such as trichloroacetic acid. It affords a convenient method for the preparation of amines from acids according to the following scheme:

$$R-COOH + H-N_3 \xrightarrow{H_2SO_4} R-NH_2 + CO_2 + N_2$$

This is a one step reaction and thus avoids isolation of intermediates; the yields often are higher than by other methods. The mechanism proposed is that the sulfuric acid activates the hydrazoic acid which adds to the carbonyl. The transient adduct then loses nitrogen to give an intermediate which under the reaction conditions is converted to an isocyanate. The isocyanate decomposes to yield carbon dioxide and the desired amine. That an isocyanate is an intermediate in the reaction was recently shown by Rutherford and Newman (60). An isocyanate was isolated by these authors when 4 - phenanthroic acid was treated under the Schmidt conditions

making use however of trifluoroacetic acid and trifluoroacetic anhydride instead of sulfuric acid as a reaction medium.

The Curtius, Hofmann, and Schmidt reactions are in that order decreasingly mild and flexible (40). All three can be carried out under anhydrous conditions, but only in the Hofmann and Curtius reactions can an anhydrous alcohol be used as a solvent. The Curtius reaction lends itself to the preparation of isocyanates, urethanes, and amines with a wide choice of experimental conditions. The Hofmann reaction is used to prepare the same type compounds but the reaction conditions are more restricted. The Schmidt reaction on carboxylic acids has been applied primarily in preparation of amines.

Some alpha substituted amino acids and methods of synthesis are given in Table VI.

TABLE IV

Alpha Amino Acids Synthesized and Methods of Synthesis

∝ - Substituted Amino Acid	Decomposition Point	Yield	Method of Synthesis	Reference
∝- Methylalanine	260°	30%	Strecker	(41)
∝- Methylphenyl-alanine	265°	40%	Strecker	(42, 43)
"	225°	58%	Method described by Bucherer and Lieb (45) Hydantoin was hydrolyzed with 60% sulfuric acid and sulfate removed with barium carbonate	(44)
∝- Amino isobutyric acid	280°	92% (from esters)	Same as above	ibid
∝- Methyl - ∝ - amino-isocaproic acid	203°		Same as above	ibid
∝- Methylvaline			Strecker	(46)

TABLE IV (continued)

α- Substituted Amino Acid	Decomposition Point	Yield	Method of Synthesis	Reference
α- Methylaspartic acid	230°	51%	Strecker synthesis with the exclusion of all alkali metals, the presence of which interfered with the precipitation of the amino acid.	(47, 48)
α- Methylleucine			Strecker	(49)
α- Methylleucine	295°		Zelinsky and Stadnikoff (51).	(50)
α- Methylthreonine	220°	45%	Tiglic acid was mixed with an excess of hypochlorous acid. The resulting solution was saturated with ammonia gas. The acid was precipitated with silver and the silver removed with hydrogen sulfide.	(52)

TABLE IV (continued)

∝- Substituted Amino Acid	Decomposition Point	Yield	Method of Synthesis	Reference
∝- Methyltryptophan			Strecker	(53)
∝- Methyltryptophan	198° - 203°		The appropriate gramine was condensed with ethyl ∝- nitropropionate followed by reduction and saponification.	(54)
∝- Methylserine			Hofmann	(55)
"	243°	64.5%	Oxidation of the corresponding oxazoline carboxylic acid with permanganate.	(56)
∝- Ethylserine	265°	75%	Same as above	ibid
∝- Methylcystine	260°	50%	Strecker (according to the method of K.T. Potts) (57).	(1)
				(58)

TABLE IV (continued)

α-Substituted Amino Acid	Decomposition Point	Yield	Method of Synthesis	Reference
α- Methylglutamic Acid	170°	85%	Strecker	(59)
α- Phenylglutamic Acid	205°	20%	Hydrolysis of Acetoamide - α - phenylglutaronitrile, prepared by cyanoethylation of α - acetamidobenzyl cyanide.	(66)
α- Methylmethionine	134°	32%	Strecker	(57)
α- Methyltyrosine	330°	35%	Strecker	ibid

CHAPTER III

PROPOSED SYNTHESIS OF ∝ - PHENYL CYSTINE

Since ∝ - methyl - DL - cystine was synthesized by Arnstein (1), using the modified Strecker reaction of Potts (57), and in this laboratory by a similar procedure (6), it was, therefore, decided to utilize this procedure for the synthesis of ∝ - phenyl cystine (Figure 5). Phenacyl chloride (II) was reacted with the sodium derivative of ∝ - toluenethiol (I) to give benzylmercapto-acetophenone (III). The cyanaminolysis of this compound proved unsuccessful (Experimental A).

It was then decided to attempt the synthesis by the Schmidt reaction (Figure 6). Sodium ethyl - ∝ - phenyl - ∝ - cyanoacetate (VIII) was to be reacted with benzyl chloromethylsulfide (IX) to give ethyl - ∝ - benzyl-mercapto - ∝ - phenyl - ∝ - cyanopropionate (X). This was to be converted to the acid and the Schmidt reaction performed in a trifluoroacetic acid - trifluoroacetic anhydride medium (60). It was decided to first attempt the synthesis of ∝ - amino - ∝ - phenylpropionic acid as this compound had similar steric arrangement to the desired compound and has already been synthesized by another method (42) (Figure 7). However, no satisfactory results were obtained (Experimental B).

Since the Strecker and the Schmidt reactions failed to give the desired product, it was decided to attempt the Curtius reaction modified by Gagnon *et al.* (39) who synthesized several ∝ - amino acids by this method. Again, the synthesis of ∝ - amino - ∝ - phenylpropionic

acid was to be used as a model compound (Figure 9). However, difficulty was encountered in the formation of the urethane and decomposition of the azide (Experimental C).

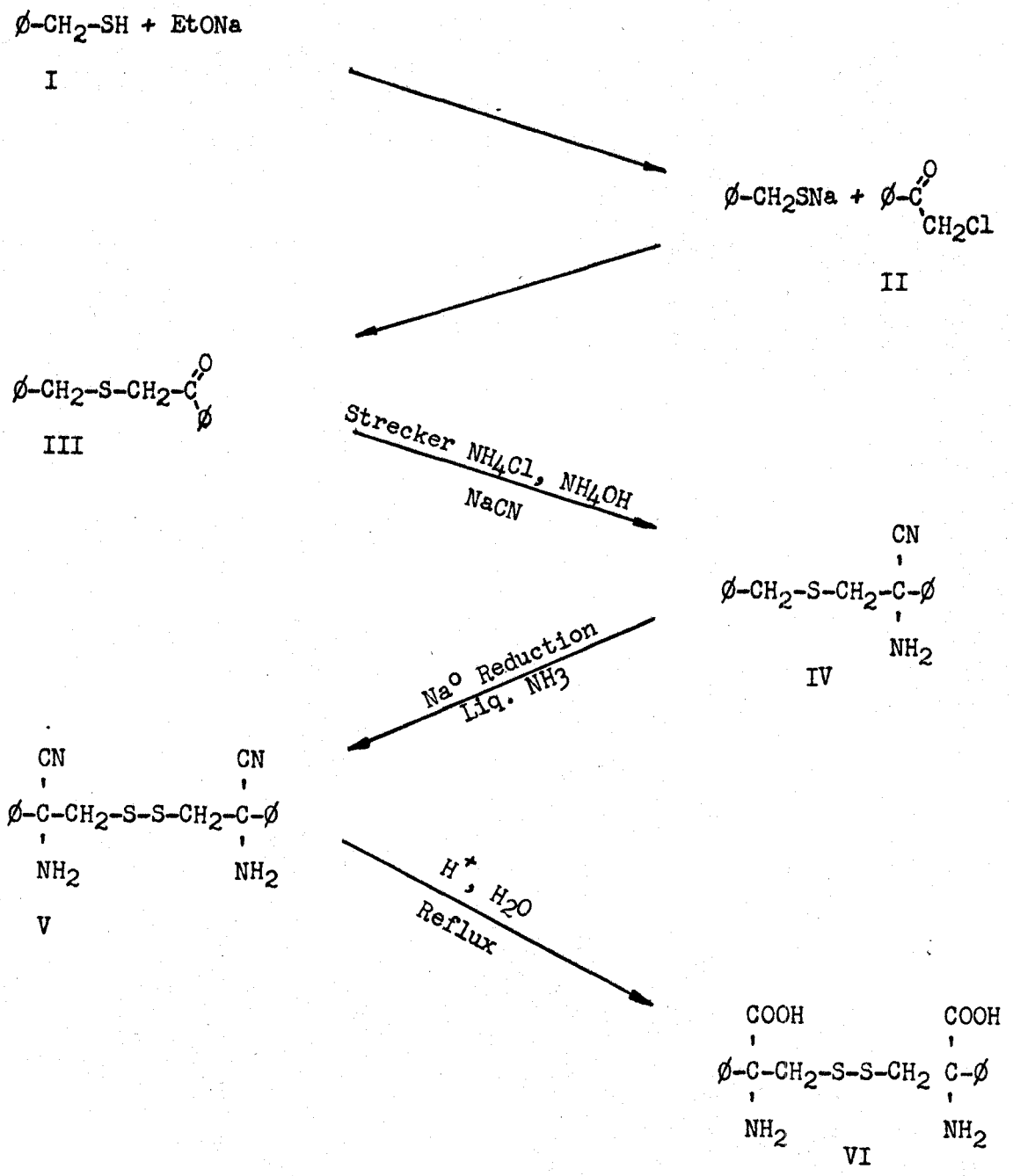


FIGURE 5

PROPOSED SYNTHESIS OF α-PHENYL CYSTINE BY STRECKER REACTION

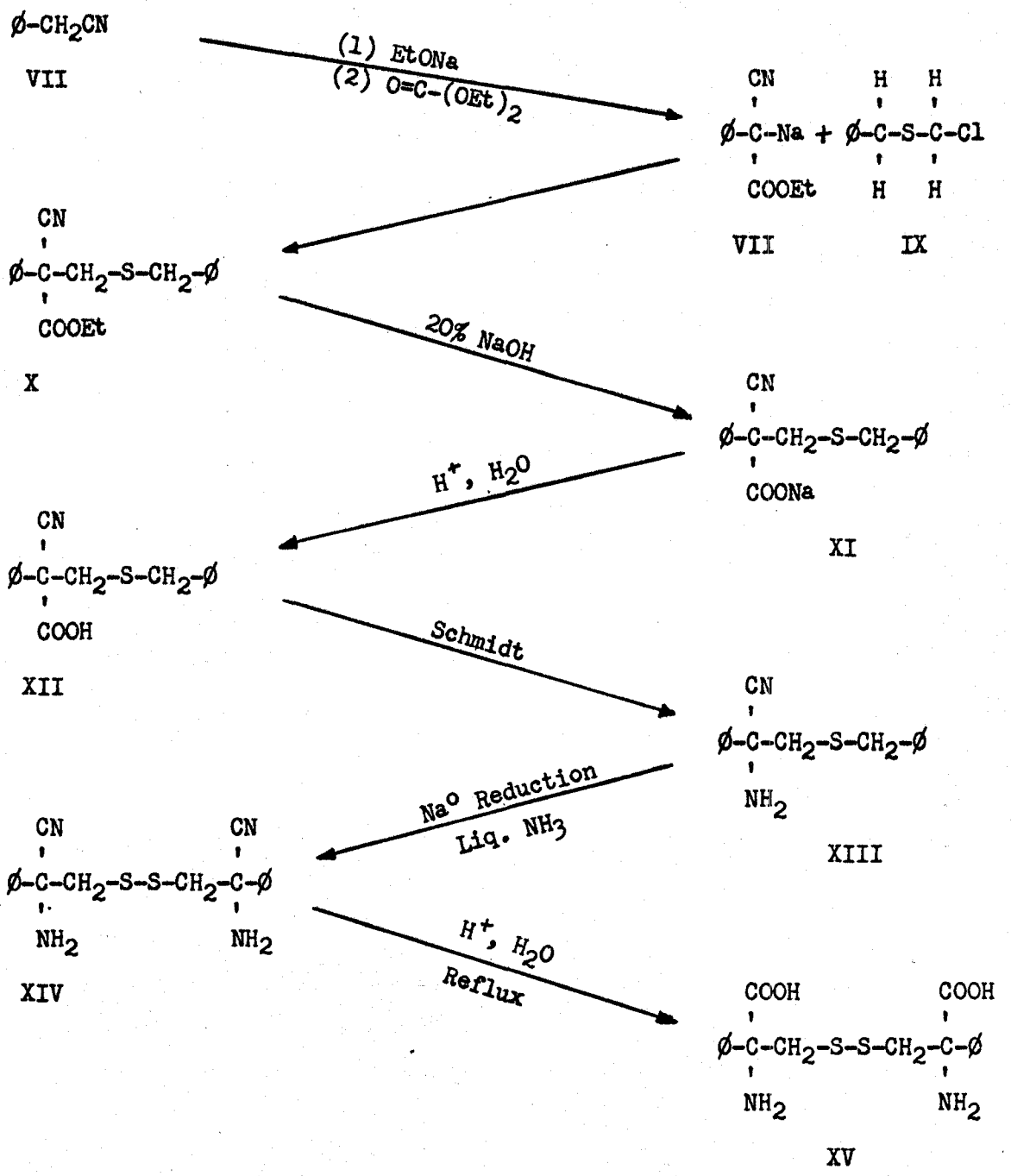


FIGURE 6

PROPOSED SYNTHESIS OF ∝- PHENYL CYSTINE BY SCHMIDT REACTION

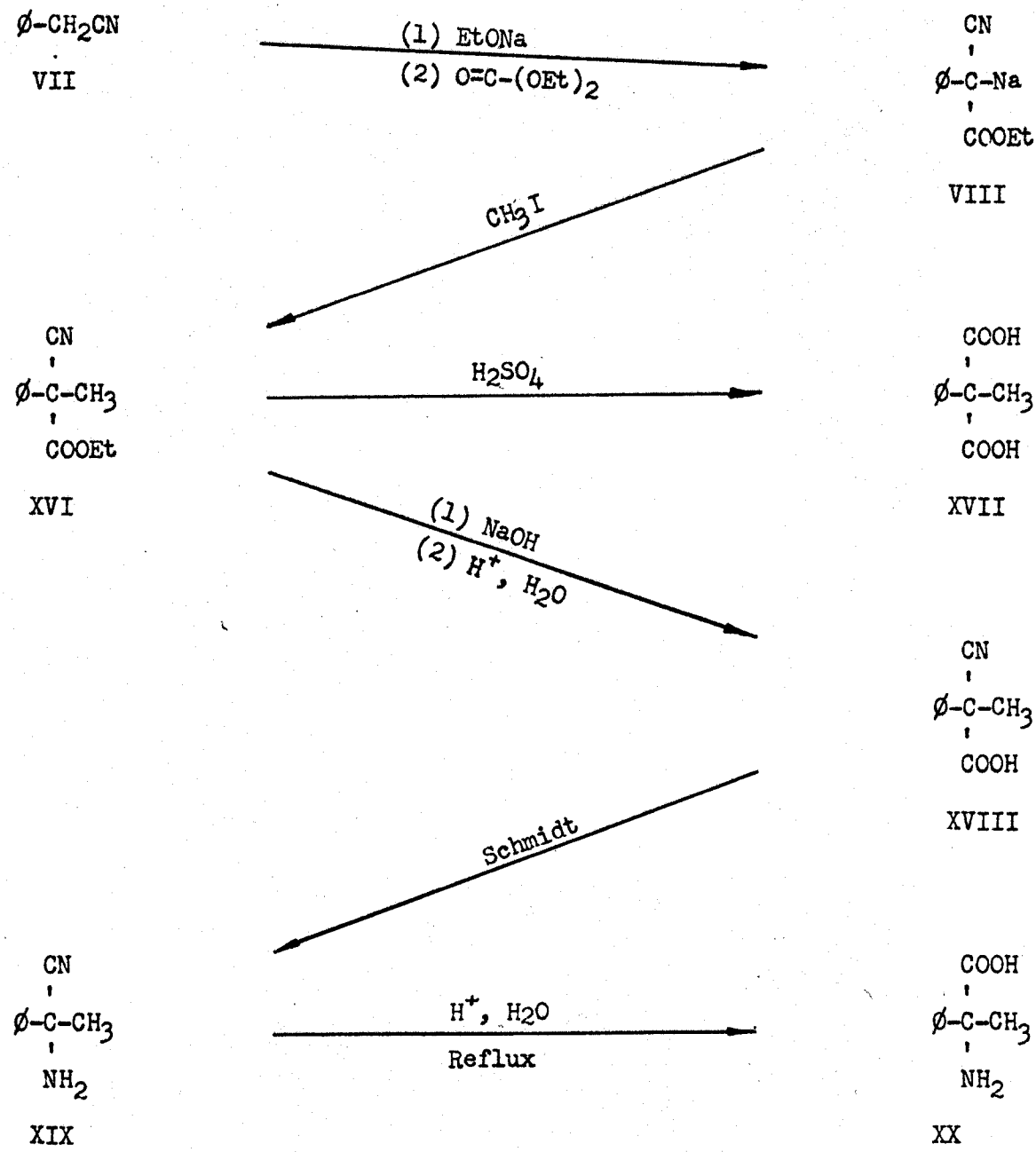


FIGURE 7

PROPOSED SYNTHESIS OF α-AMINO-α-PHENYL-PROPIONIC ACID BY SCHMIDT REACTION

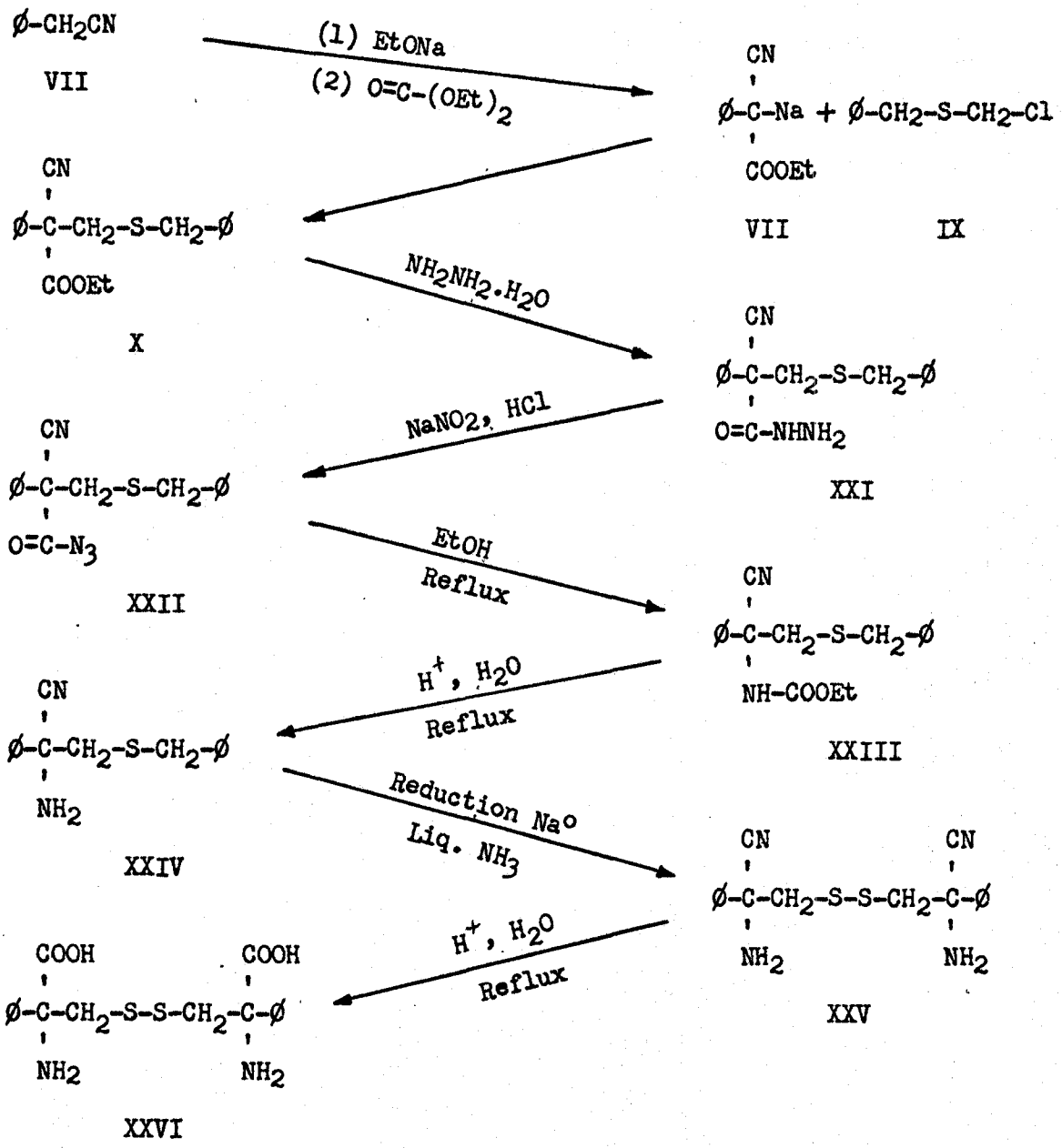


FIGURE 8

PROPOSED SYNTHESIS OF α- PHENYL CYSTINE BY CURTIUS REACTION

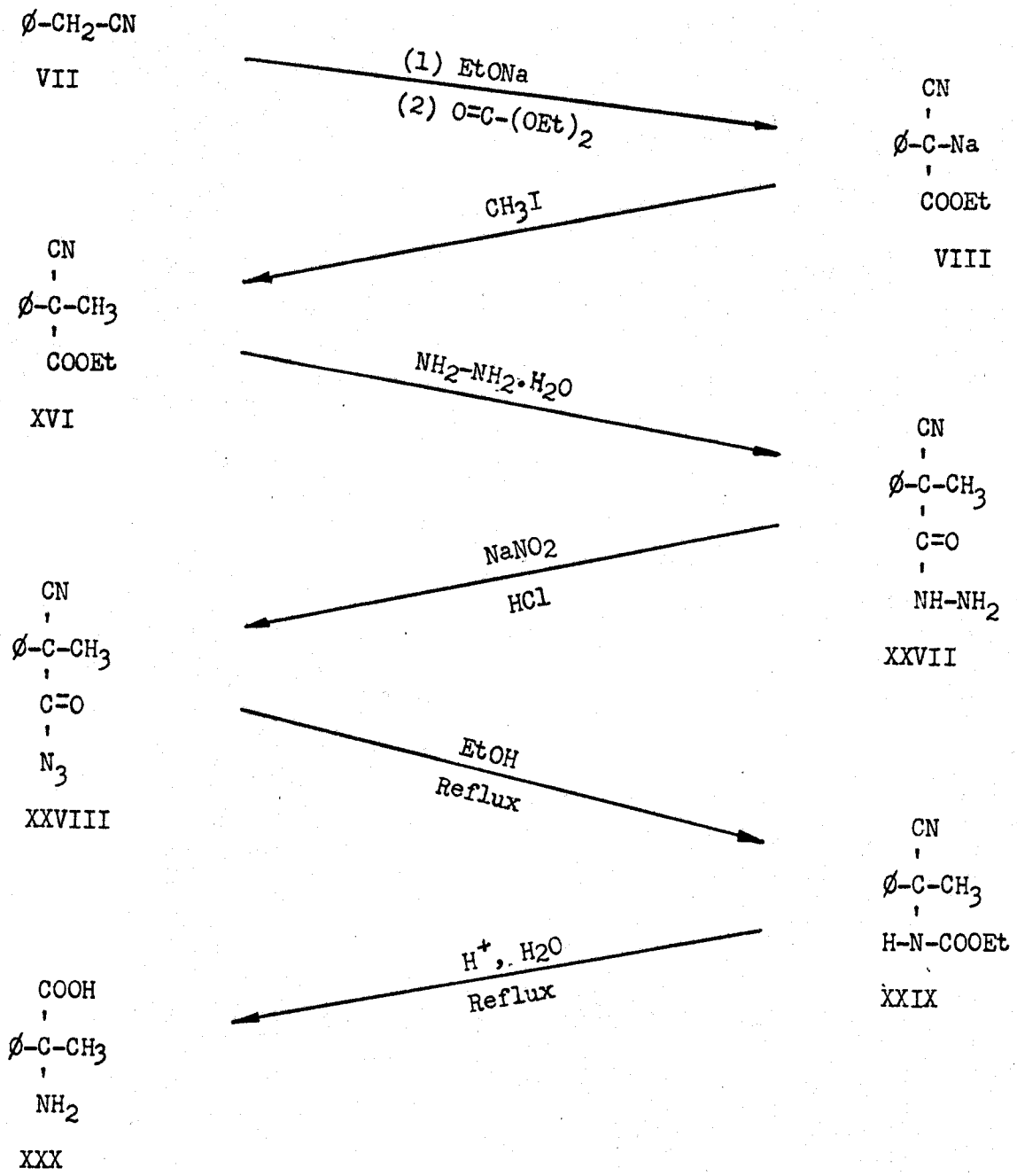


FIGURE 9

PROPOSED SYNTHESIS OF ∝-AMINO-∝-PHENYLPROPIONIC ACID BY CURTIUS REACTION

CHAPTER IV

EXPERIMENTAL A

Benzylmercaptoacetophenone (III)

Sodium ethoxide was prepared by treating 1.56 g. of metallic sodium with 35 ml. of absolute ethanol, at 0°C. To this solution α - toluenethiol (I) (7.9 g. - 0.066 mole) was added with constant stirring. The temperature rose to about 60°C and the mixture turned amber in color. After 30 minutes, while stirring, phenacyl chloride (II) (7.73 g. - 0.05 mole) was added in one portion. The temperature rose to 80°C. The orange colored mixture was stirred and cooling was effected by means of an ice-water bath. Crystallization resulted after approximately ten minutes of standing. Within thirty minutes the entire reaction mixture solidified. Ethanol (25 ml.) was added to increase the mobility of the mixture, which was then added to water (150 ml) and thoroughly agitated. The light orange benzylmercaptoacetophenone was recovered by filtration and air dried. The precipitate, recrystallized from absolute ethanol yielded 9.8 g. (61.2%) colorless needles m.p. 89°C - recorded 89°C (67), (2,4, - DNP m.p. 168-169°C).

The yields in subsequent runs were increased to 95% by controlling the temperature in all phases of the reaction from 0-5°C by means of an ice-water bath.

β - Benzylmercapto - α - Amino - α - Phenylpropionitrile (IV)

(a) Sodium cyanide (12.5 g. - 0.25 mole, 98% purity) dissolved in 25 ml. water and ammonium chloride (14.5 g. -

0.25 mole) dissolved in 35 ml. of water was poured into a single-necked 500 ml. round-bottomed flask which contained 33.5 ml. (0.25 mole) of concentrated ammonium hydroxide. The solution was agitated while benzylmercaptoacetophenone (III) (30.2 g. - 0.125 mole), dissolved in 75 ml. of 95% ethanol, was added. The flask was stoppered with a rubber stopper, which was wired in place, and the mixture was stirred for 16 hours. The heterogeneous mixture was poured on a mixture of ice and hydrochloric acid. A precipitate formed which on recrystallization from ethanol yielded colorless needles (m.p. 89°). A mixture melting point determination with an authentic sample of benzylmercapto-acetophenone yielded no depression. The recovery of starting material on several runs, with increased stirring time from 24 to 72 hours, was approximately 92%.

(b) A procedure similar to that described above was attempted with the following modification. The mixture was held at 60°C with stirring for times varying from 24 to 72 hours. Again starting material was recovered almost quantitatively (98%).

(c) The above procedure was again employed with the addition of dioxane (250 ml.) and double amounts of sodium cyanide and ammonium chloride. The mixture was then saturated with ammonia gas and kept at 60°C with stirring for 16 hours. The solution was poured on a mixture of ice and hydrochloric acid. Starting material was recovered quantitatively.

(d) Benzylmercaptoacetophenone (30.2 g. - 0.125 mole) was added to a 500 ml. round-bottomed flask which

contained an ethanolic solution (300 ml.) of 12.5 g. (0.25 mole) of sodium cyanide and 14.5 g. (0.25 mole) of ammonium chloride. The flask was sealed and the mixture agitated at 60°C in a water bath for 20 hours. The contents were then poured on a mixture of ice and hydrochloric acid. Recovery of starting material was 98%.

(e) Benzylmercaptoacetophenone (6.0 g. - 0.025 mole) in 200 ml. of ether was added to a 300 ml. pyrex gas pressure bottle which contained a solution 6.4 g. (0.13 mole) of ammonium chloride and 6.0 g. (0.13 mole) of sodium cyanide in 50 ml. of water. The bottle was stoppered with a rubber stopper which was held in place with adhesive tape. The bottle was then put on a shaker for 48 hours. The etheral layer was separated and dried with calcium chloride. After the removal of the calcium chloride the ether was removed in vacuo. The residual precipitate, weighing 6.0 g. after recrystallization from ethanol, was found to be starting material by a mixture melting point determination.

(f) Benzylmercaptoacetophenone (6.0 g. - 0.025 moles) was added to a 300 ml. pressure bottle containing a solution of absolute alcohol (250 ml.) saturated with dry ammonia gas and hydrogen cyanide. The hydrogen cyanide gas was produced by reacting 12 g. (0.5 mole) of sodium cyanide with 1:1 sulfuric acid under a well ventilated hood. The reaction mixture was then treated with dry ammonia gas until saturated. The pressure bottle was sealed and placed on the shaker for 20 hours. The contents were poured on a mixture of ice and hydrochloric acid. Starting material was obtained again (92% recovery).

(g) Benzylmercaptoacetophenone (6.0 g. - 0.025 mole) was dissolved in a solution of dimethyl formamide (200 ml.) containing 6.0 g. (0.13 mole) of sodium cyanide and 6.2 g. (0.13 mole) of ammonium chloride. The solution was then saturated with dry ammonia gas. The bottle was sealed and shaken for 72 hours. Upon analysis 90% of the starting material was obtained.

NOTE: All the preceding methods were attempted with various modifications without any observable results. Therefore, it was decided to attempt another approach.

EXPERIMENTAL B

Benzyl Chloromethylsulfide IX

(a)α-Toluenethiol[1] (I) (9.94 g. - 0.08 mole) was added slowly with constant stirring to a single-necked 100 ml. reaction flask containing a solution of sodium ethoxide prepared by reacting 1.8 g. of metallic sodium with 25 ml. of absolute alcohol. Heat was evolved but the reaction mixture was quickly cooled with an ice-water bath. The resulting solution was kept at approximately $0^{o}C$ while dichloromethane (6.0 g. - 0.09 moles) was added with stirring. After an hour a fine white precipitate of sodium chloride was formed on the bottom of the reaction vessel. The precipitate was removed by filtration and the excess ethanol was removed in vacuo. A white crystalline substance remained. This substance was recrystallized from

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1 Rubber gloves and the usual protection of a laboratory coat is essential as α- toluenethiol and benzyl chloromethyl sulfide have a strong disagreeable odor which penetrates and lingers in the skin and clothing.

benzene, to yield 6.1 g. of product (m.p. 44.8°C). This did not have the physical properties of the desired material, which is a colorless liquid, b.p. 120-125° at 15 mm., the same crystalline substance was always obtained. It was not considered expedient at the time to identify this crystalline material.

(b) Since the above procedure failed it was decided to use the method of Böhme and Fischer (61). Dry hydrogen chloride was bubbled through a mixture of α - toluenethiol (62.1 g. - 0.5 mole) and 15.0 g. (0.5 mole) of para-formaldehyde. The mixture was shaken continually and held at temperatures between -15 to -5°C throughout the reaction. After 2 hours the solution became semi-solid and would not absorb any more dry hydrogen chloride. Anhydrous calcium chloride was added and the mixture was left to stand for twenty-four hours in a flask closed with a calcium chloride drying tube. The aggregate was filtered and the filtrate was distilled in vacuo under anhydrous conditions at 15 mm. pressure. Benzylchloromethyl sulfide was obtained in the fraction 120-125°C with a 20% yield and a refractive index at 21°C of 1.5775. This yield was quite different from the 60% reported by Böhme and Fischer, but despite repeated runs in smaller quantities the yields never increased above 30%.

(c) Because the yields were so poor for the method described in (b); variations of this procedure were tried, and better results were obtained with the following procedure:

α - Toluenethiol (12.0 g. - 0.08 mole) was added to a mixture containing 5.0 g. of anhydrous calcium

chloride and a large excess of (6.0 g.) of paraformaldehyde in 60 ml. of benzene. This mixture was then treated with dry hydrogen chloride[2] with constant stirring while the temperature was held between -15 and -5°C. The dry hydrogen chloride was added until the mixture became solid (approximately 1.5 hours). More anhydrous calcium chloride was added and the mixture was allowed to rise to room temperature under anhydrous conditions.[3] After 24 hours the solid was removed by filtration. The benzene was removed in vacuo from the filtrate. The resulting liquid was distilled in vacuo at 15 mm. Benzylchloromethyl sulfide was collected in 55% yield, m.p. 120-125°C; n_D^{20} 1.5780.

Sodium Phenylcyanoacetate (VII)(62)

Sodium ethoxide was prepared from 12.0 g. (0.52 mole) of sodium and 300 ml. of anhydrous ethanol in a 1 liter three-necked flask fitted with a reflux condenser in which was placed a calcium chloride tube.

The anhydrous alcohol was prepared from commercial grade absolute alcohol according to Fieser (63). A mixture of 5 g. of magnesium turnings, 60 cc. of absolute alcohol and 0.5 g. of iodine were heated to reflux temperature in a large flask until a vigorous reaction ensued and until the magnesium was nearly all converted into the ethoxide.

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2 The dry hydrogen chloride was added very slowly as much heat was evolved at this stage and the temperature very easily rose above -5°.

3 This solution should not be allowed to stand for more than 24 hours as beyond this period of time a secondary reaction becomes predominant and difficulty was experienced in distillation as well as in obtaining a reasonable yield.

Nine hundred ml. of absolute alcohol were then added. The reaction mixture was then boiled for an additional hour after which the anhydrous alcohol was removed by distillation 78.4°C.

After the sodium had reacted completely, a condenser was arranged for distillation under reduced pressure and the excess ethanol was removed via a steam bath. Sodium ethoxide was obtained as a fine white powder.

As rapidly as possible after removal of the ethanol, the flask was fitted with a rubber-sealed stirrer, a dropping funnel, a distilling head containing a thermometer and a condenser arranged for distillation into a flask protected from moisture by a calcium chloride tube. Then 300 ml. of dry diethyl carbonate, 80 ml. of dry toluene and 58.59 g. (0.50 mole) of phenylacetonitrile (VII) were added in that order. While the contents were stirred vigorously the flask was heated and the cake of sodium ethoxide dissolved. After distillation started, dry toluene was added dropwise at approximately the same rate that the distillate was collected. Approximately 200 - 250 ml. of toluene were added over a period of 2 hours.

Any ethanol which remained in the sodium ethoxide together with the ethanol produced during the reaction was removed during this period. The progress of the carbethoxylation reaction was followed by temperature readings. During the first half of the heating period distillation occurred with vapour temperatures of 80 - 85°C. As the reaction neared completion and the ethanol was removed the temperature rose to 110 - 115°C. Near the end of the reaction, the sodium salt of ethyl phenylcyanoacetate (VII) precipitated as a white crystalline solid.

Ethyl - ∝ - Cyano - ∝ - Phenylpropionate (XVI)

The above mixture of ethyl phenylcyanoacetate and toluene was cooled to room temperature and 0.5 mole of methyl iodide (mixed in approximately 200 mls. of toluene, was added dropwise with vigorous stirring. When the addition was complete the mixture was allowed to be stirred continuously five to eight days. The reaction mixture was tested with Hydrion paper from time to time. After 4 days the solution was neutral and this was taken as the end point of the reaction. The resultant amber coloured mixture contained a white precipitate. The mixture was acidified with dilute sulfuric acid and then water (300 ml.) was added with vigorous stirring to dissolve the inorganic salt. The top organic layer was separated and the bottom aqueous layer was extracted 3 times with ether. The top layer was combined with the etheral extracts and dried over anhydrous sodium sulfate. The ether and toluene were then removed in vacuo. The residue was distilled at 8 mm. pressure. Ethyl - ∝ - cyano - ∝ - phenylpropionate was collected at 134°C with a 72.7% overall yield (n_D^{18} 1.4955).

It was later found that with the addition of approximately 10% excess of methyl iodide, while the reaction mixture was held at 40°C by means of a water bath, that the reaction time decreased by one-half. The water and the ether were removed in vacuo without prior treatment with anhydrous sodium sulfate. The modifications increased the overall yield of the ester to 91%;

Analysis:	C	H	N
Calculated:	70.91%	6.45%	6.89%
Found:	70.80%	6.45%	6.96%

∝ - Cyano - ∝ - Phenylpropionic Acid (XVIII)

Ethyl - ∝ - cyano - ∝ - phenylpropionate (XVI) (88.0 g. - 0.5 mole) was added to 10% sodium hydroxide (500 ml.) and shaken (one hour) until a clear solution was obtained. The solution was extracted with ether to remove all unsaponified starting material. The residue was poured with vigorous stirring into a mixture of concentrated hydrochloric acid and cracked ice. A heavy white precipitate was formed which was removed by filtration and dried. Melting points were determined and found to vary from 62 - 98°C showing the presence of impurities.

The precipitate was thoroughly washed in ice cold water and air dried. The dried acid was then dissolved in a minimum amount of chloroform and dried over sodium sulfate. The sodium sulfate was removed and the acid was recrystallized from petroleum ether. The yield was almost quantitative (98%, m.p. 99.5 - 100°C).

The molecular weight of the acid was determined by titrating a weighed amount of ∝ - cyano - ∝ - phenyl-propionic acid dissolved in 75 ml. of water and 10 ml. of ethanol with a standard sodium hydroxide solution:

Found	176.0 g.
Calculated	175.2 g.

Precautions had to be taken in drying and weighing the sample as it was found to be fairly hygroscopic and usually existed as the monohydrate. This was evident when dried ∝ - cyano - ∝ - phenylpropionic acid, was allowed to stand in the atmosphere for a short time. By analysis the molecular weight was then found to be 193.8 g. (Calculated monohydrate: 192.3).

α - methyl - α - Phenylmalonic Acid (XVII)

Ethyl - α - cyano - α - phenylpropionate (XVI) (5.00 g.) was dissolved in 10 ml. sulfuric acid and heated on a steam bath for 1.5 hours. The ester was again treated with 10 ml. of sulfuric acid and heated for another hour. Water was slowly added (5 ml. at a time) every twenty minutes while heating was continued on the steam bath. This treatment was carried out for 2 hours during which time a white precipitate began to form. The reaction mixture was poured on ice and the precipitate was isolated by filtration, washed and air dried. A 52.5% yield was obtained, m.p. 157°C. This agrees with the value reported in Beilstein (65).

α - Amino - α - Phenylpropionitrile (XIX)(60)

α - Cyano - α - phenylpropionic acid (XVII) (5 g. - 0.028 mole) was dissolved in a 100 ml. solution containing equal volumes of trifluoroacetic acid and trifluoroacetic anhydride in a 250 ml. erlenmeyer flask. The flask was stoppered with an anhydrous calcium chloride drying tube and cooled in an acetone dry ice bath to -5°C. A large excess of sodium azide (3 g.) was added portionwise with stirring. A reaction seemed to occur because of the liberation of gas. After an hour the mixture was poured on ice and a precipitate was obtained. The starting material was recovered almost quantitatively.

Modifications of this method were attempted by varying the reaction temperature, the ratio of the trifluoroacetic acid to trifluoroacetic anhydride, and the amounts of sodium azide. Each attempt yielded starting material almost quantitatively as shown by mixture melting points.

EXPERIMENTAL C

α- Cyano - α - Phenyl Propionylhydrazide (XXVII)

Ethyl - α - cyano - α - phenylpropionate (XVI) (101.0 g. - 0.5 moles) was added slowly with stirring to an excess of 100% hydrazine hydrate (30.0 g. - 0.6 mole). The reaction produced a considerable amount of heat and after a time the two layers became miscible. The solution was allowed to stand for 16 hours during which time it solidified. The resultant mixture was subjected to filtration and the precipitate was washed with cold water. The precipitate was recrystallized from absolute ethanol yielding a white crystalline substance (m.p. 185°C). The yield was 76%. In subsequent runs absolute ethanol was added to the reaction mixture to increase its mobility. This increased mobility along with a rigid control of the reaction temperature increased yields from 75 - 95%. Yields were also increased through recovery from the mother liquor.

Analysis:	C	H	N
Calculated:	63.48%	5.85%	22.26%
Found:	63.46%	5.79%	22.09%

α- Cyano - α - Phenyl Propionylazide (XXVIII)

α- Cyano - α - phenyl propionylhydrazide (XXVII) (8.0 g. - 0.25 mole) was dissolved in 15% hydrochloric acid (100 ml.), cooled to 0°C by means of an ice-water bath, and covered with a layer of diethyl ether. Sodium nitrite (5.0 g. dissolved in 150 ml. of water) was added dropwise with vigorous stirring. Caution was taken that the temperature of the reaction mixture never rose above 10°C. The azide formed immediately as a dark yellow gummy

substance which stuck to the flask and the stirrer. Upon the addition of all the sodium nitrite solution the azide was removed by filtration and washed with several small portions of cold water. The azide was not very soluble in ether or ethanol which is irregular compared to the properties of other azides reported in the literature (64). The azide precipitate was air dried. The yields were 65-70% (m.p. 160-80°C).

In the crude form the azide was a dark yellow powder which was insoluble in the more common organic solvents except dimethylformamide. Purification was effected by dissolution of the compound in dimethylformamide and recrystallizing it by the addition of water. Some success in the recrystallization of the azide was obtained with acetone but very little success from ethanol. Recrystallization from ethanol produced a light, bright yellow powder (m.p. 180°C).

Analysis:	C	H	N
Calculated:	59.99%	4.03%	27.98%
Found:	59.53%	5.17%	24.16%
	59.55%	5.10%	23.85%

By synthesizing the azide at 20°C it was observed that the product was more crystalline and the yields increased.

Ethyl N -(1 - cyano - 1 - phenyl ethyl) Carbamate (XXIX)

α - Cyano - α - phenyl propionylazide (XXVIII) (10.0 g. - 0.05 mole) was added to a 200 ml. reaction flask containing absolute ethanol. This mixture was heated at reflux temperatures for 24 hours and then poured on cracked ice. A precipitate formed, but upon analysis it was shown to be starting material. Variations of this method were attempted. More ethanol was used; longer periods of boil-

ing were employed; and different solvents such as toluene, methanol, and benzene were used but the urethane was not obtained.

Decomposition of the Azide to the Acid

∝- Cyano - ∝ - phenyl propionylazide (XXVIII) (10.0 g. - 0.05 mole) was added to a 100 ml. flask containing 50 ml. of absolute ethanol. The solution was saturated with dry hydrogen chloride (during which time the mixture became warm and the azide dissolved) and heated at reflux temperatures until the evolution of gas subsided. The alcohol was removed under reduced pressure and a white solid precipitated. Upon analysis it was found to be ammonium chloride.

Several variations of this procedure were attempted but no promising results were obtained. It was then decided to decompose the azide in other ways. Small portions of azide were heated in such solvents as diethyl carbonate, xylene, toluene, and benzene, but no decomposition took place. However, decomposition did take place when a minute portion of cuprous chloride was added. A solid was obtained in very low yields (m.p. 147-150°C).

The azide decomposed in ethanol and methanol when heated with small amounts of sulfuric acid. The rate seemed to depend on the concentration of the acid. A small amount of yellowish oil was obtained. All these decompositions gave products with too small a yield to bear further investigation.

The azide was found to decompose in both dimethylformamide and tetramethyl urea at temperatures from 110-125°C. The product obtained was a yellow precipitate (m.p. 135°C). Infrared studies of these decomposition

products showed evidence of carbon nitrogen single bonds and carbon nitrogen double bonds.

SUMMARY AND DISCUSSION

Benzylmercaptoacetophenone (III) was obtained in yields of 95% by controlling the temperature in all phases of the reaction from 0 - 5°C. The cyanoaminolysis of this compound following the modified Strecker reaction of Potts (57) was unsuccessful as quantitative recovery of the starting material was always obtained. Since there is a steric effect in this reaction, which might be overcome by increasing the reaction time, the reaction was run by increasing the time five fold. Starting material was again recovered quantitatively.

A similar procedure was then employed with the reaction mixture being held at 60° for 72 hours attempting to overcome the steric effect by increasing the energy in the system. However, this also proved to be unsuccessful. Dioxane, with doubled amounts of sodium cyanide and ammonium chloride, was employed to increase the solvolysis of the system and orientation of the reactants, but this also was unsuccessful.

A completely anhydrous system was employed, with absolute ethanol saturated with ammonia, without success. Absolute ethanol was employed, saturated with ammonia and hydrogen cyanide, again without success. Dimethylformamide was then used in place of ethanol as it has a higher dielectric constant, this also proved unsuccessful. The Strecker method was abandoned as the steric effect seemed to be too great for the reaction to proceed.

It was decided to attempt the synthesis by the Schmidt reaction. Sodium ethyl - α - phenyl - α - cyano-acetate (VIII) was to be reacted with benzyl chloromethyl-

sulfide (IX) to give ethyl - β - benzylmercapto - α - phenyl - α - cyanopropionate (X). This was to be converted to the acid and the Schmidt reaction performed to yield the amino acid. The benzyl group was then to be cleaved by sodium - liquid ammonia reduction.

The synthesis of benzyl chloromethylsulfide (IX) was first attempted by reacting α- toluenethiol with sodium ethoxide to yield the sodium salt. This salt was reacted with methylene chloride giving a product, which, on purification yielded a white crystalline compound. This did not have the physical properties of the desired material which is a colorless liquid. In spite of different attempts the same crystalline compound was obtained. It was not considered expedient at the time to identify this compound.

The method of Böhme and Fischer (61) was then employed with varying results. The yield obtained was about half of what was reported. However, by varying this procedure the yields were increased to 55%.

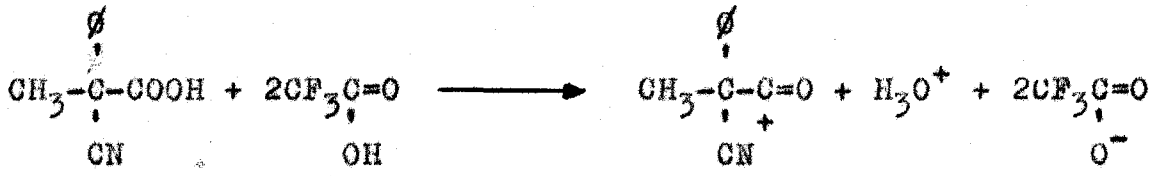
At this point it was decided to first attempt the synthesis of α- amino - α - phenylpropionic acid as this compound has similar steric arrangement to the desired compound and has already been synthesized by another method.

Sodium phenylcyanoacetate (VII) was synthesized according to Horning and Finelli (62) and alkylated with methyl iodide to give ethyl - α - cyano - α - phenyl-propionate (XVI). The corresponding acid was formed by saponifying the ester and hydrolyzing the sodium salt with an ice-hydrochloric acid mixture. The purified acid, upon analysis, was found to exist as a monohydrate.

α- Methyl - α - phenylmalonic acid (XVII) was obtained by hydrolyzing the cyanoester with concentrated sulfuric acid and subsequent addition of water.

The Schmidt reaction was attempted with α - cyano - α - phenylpropionic acid in a trifluoroacetic acid - trifluoroacetic anhydride medium according to Newman and Rutherford (60). However, no satisfactory results were obtained although several modifications of the method were attempted. Gas was liberated which on analysis proved to be nitrogen, and starting material was always recovered quantitatively.

Evidently the nitrogen arose from the decomposition of hydrazoic acid rather than from the Schmidt intermediate. The failure of the Schmidt reaction under these conditions may well be a result of the inability of the starting cyanoacetic acid to form a stable acyl-oxo carbonium ion according to the following equation:



The formation of acyl-oxo carbonium ions from the reaction of concentrated sulfuric acid with sterically hindered aromatic acids has long been established by Newman (68). For example, the esterification of mesitoic acid is possible only by treatment of the acid first with 100% sulfuric acid prior to treatment with alcohol. It was hoped that the same steric forces which allow hindered aromatic acids to form transient acyl-oxo carbonium ions would be operative in this case of an aliphatic system. Evidently other factors are involved.

Since the Strecker and the Schmidt reactions failed to yield the desired compound, it was decided to attempt

the Curtius reaction modified by Gagnon et al. (39).

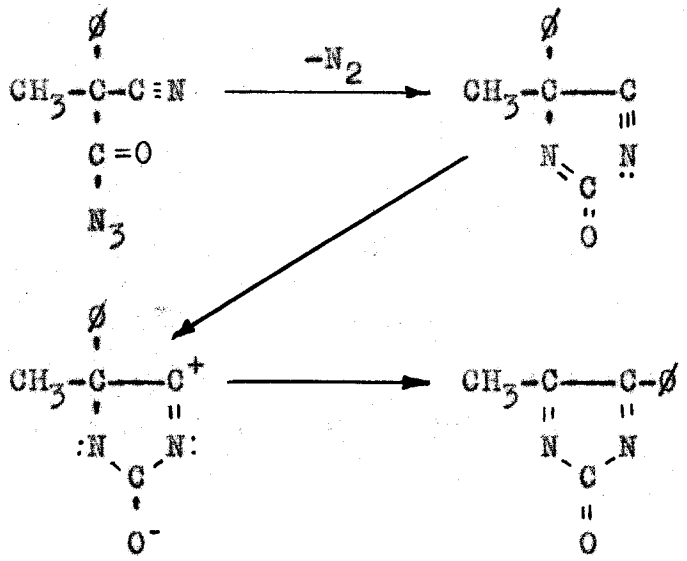
The synthesis of α - amino - α - phenylpropionic acid was again attempted and used as a model compound. α - Cyano - α - phenyl propionylhydrazide (XXVII) was obtained in good yields by reacting ethyl - α - cyano - α - phenylpropionate with an excess of 100% hydrazine hydrate. The corresponding azide was obtained by reacting the hydrochloric acid salt of the hydrazide with nitrous acid below 5°C. The yields were good but there were discrepencies on analyses of hydrogen and nitrogen from the theoretical calculations.

The synthesis of the corresponding urethane was attempted but only starting material was recovered. Different solvents such as toluene, methanol, and benzene were used but the urethane was not obtained.

Decomposition of the azide was then attempted. Given amounts of azide were decomposed in ethanol and methanol with hydrochloric and sulfuric acid as catalysts but yields were low. Decompositions were also attempted in diethyl carbonate, xylene, toluene, and benzene with small traces of cuprous chloride. All these decompositions gave products with too small a yield to bear further investigation.

The azide was found to decompose in both dimethyl-formamide and tetramethyl urea yielding a product which upon infrared studies showed evidence of carbon nitrogen double bonds.

It is conceivable that the azide rearranged to the corresponding isocyanate which then underwent an intra-molecular reaction involving the cyano function as follows:



It was decided, however, to leave this product to another investigation.

It was at this point that studies were terminated. The possibilities of the Curtius reaction being successful in synthesizing the desired compound are very good. It is suggested, however, that further investigation of the azide be done as the analysis is quite varied from what was theoretically expected. Also some of the properties exhibited by the apparent azide obtained, do not coincide with other azides reported in the literature. It is also suggested to investigate the product which was obtained from the decomposition of the azide in dimethylformamide and tetramethyl urea.

CONCLUSION

From the experimental data it may be concluded that the synthesis of ∝- phenyl cystine can not be accomplished by either the Strecker or the Schmidt reactions under the experimental conditions attempted. However, the possibility of the synthesis of this compound by the Curtius reaction has not been eliminated as further investigation in this area is advocated by the author. The successful synthesis of ethyl ∝- cyano - ∝ - phenylpropionate and ∝ - cyano - ∝ - phenyl propionylhydrazide may be reported as these compounds have not appeared in the literature.

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Polarographic Determination of Alpha-Methyl-DL-cystine

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▶ **Because of a recent synthesis of a substituted amino acid, α-methyl-DL-cystine, it became necessary to have a method of determining this compound. The polarographic determination of the substance in 0.1N hydrochloric acid, using thymol as a maximum suppressor, was investigated. The relationships of concentration of the compound and temperature to the diffusion current were studied. The influences of thymol concentration and pH on the apparent half-wave potential were determined. A linear relationship of the diffusion current to the concentration of α-methyl-DL-cystine was observed in the range of 5 × 10^{-4} to 2 × 10^{-3}M. The system is not reversible.**

A RECENT synthesis of α-methyl-DL-cystine by Arnstein (*1*), which has been confirmed in this laboratory (*8*), made it desirable to establish a method of analysis for this compound.

Cystine can be determined colorimetrically by reduction to cysteine and by using reagents which form colors in the presence of free sulfhydryl groups (*2, 9*). Cystine can also be determined polarographically, and studies of this type have been reported (*7*).

Because the compound under investigation has a structure similar to cystine, it was thought feasible to attempt a polarographic estimation.

EXPERIMENTAL

Apparatus and Materials. The Sargent (E. H. Sargent & Co.) Model XXI Polarograph was employed for this study. A Heyrovský polarographic cell was used during most of this work; an H-cell with S.C.E. was used to determine $E_{1/2}$. The capillary constant, $m^{2/3}t^{1/6}$ (m = 2.57 mg. per second; t = 3.00 seconds), was 2.253 at 25° C. and −0.555 volt. All pH measurements were made with a Beckman Model G pH meter. Buffer solutions employed were prepared according to Clark and Lubs (*3*).

The α-methyl-DL-cystine was recrystallized three times from absolute ethyl alcohol prior to use in this study. A stock solution of 1 × $10^{-2}M$ was prepared in 0.1N hydrochloric acid. A 1.2 × $10^{-3}M$ thymol solution in 0.1N hydrochloric acid was prepared for use as a maximum suppressor.

Procedure. The solutions used for analysis were prepared in a 100-ml. volumetric flask by adding the appropriate concentrations of α-methyl-DL-cystine and thymol, and diluting to volume with 0.1N hydrochloric acid or buffer.

The solutions were transferred to a polarographic cell and nitrogen (purified by passing through ammoniacal cuprous chloride) was bubbled through for 5 minutes. Polarograms were run through the range of 0.0 to −1.0 volt. The drop rate was adjusted to 3 seconds and the temperature was controlled to ± 0.1° C.

Table I. Effect of Concentration on Diffusion Current

Concn. α-Methyl-DL-cystine, Mole/Liter	Diffusion Current, Microamperes[a] 25° C.	30° C.	37.5° C.
2 × 10^{-3}	8.66	8.89	8.03
1.5 × 10^{-3}	6.42	6.78	6.03
1 × 10^{-3}	4.19	4.60	3.98
7.5 × 10^{-4}	3.64	3.53	3.06
5 × 10^{-4}	2.28	2.36	2.01

[a] Average of three determinations.

Table II. Effect of Temperature on Diffusion Current

Temperature, ° C.	Diffusion Current, μa.	
0.0	4.28	4.26
11.5	5.28	5.28
19.0	5.68	5.66
22.5	6.00	6.00
25.0	6.22	6.21
30.0	6.54	6.64
35.0	7.06	7.04
40.0	7.36	7.36
45.0	8.00	8.00

RESULTS AND DISCUSSION

Effect of Concentration on Diffusion Current. The effect of concentration of α-methyl-DL-cystine (using 1.2 × $10^{-4}M$ thymol as the maximum suppressor at 25°, 30°, and 37.5° C., respectively) on the diffusion current results in a linear relationship (Table I).

The diffusion current was measured from the top of the first wave to the top of the second wave as there occurs a prewave with thymol. This type of prewave also occurs with cystine and has been studied by Kalousek, Grubner, and Tochstein (*5*). Figure 1, curve 2, is a typical example of a polarogram obtained under the above conditions.

Except for the study of diffusion current dependence on temperature, where the height of the diffusion current represents the prewave plus the top wave, the diffusion currents and half-wave potentials are based on the top wave only wherever a prewave occurred.

The half-wave potential ($E_{1/2}$) was measured *vs.* S.C.E. at 20° C. using 1 × $10^{-3}M$ α-methyl-DL-cystine. $E_{1/2}$ was observed to be −0.555 and −0.718 volt for thymol concentrations of 1.2 × 10^{-4} and 4.8 × $10^{-4}M$, respectively.

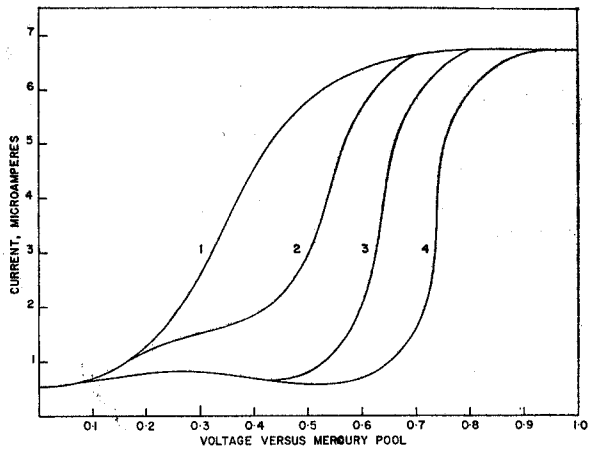

Figure 1. Effect of thymol concentration on shape of polarogram

1. $2.4 \times 10^{-5}M$
2. $1.2 \times 10^{-4}M$
3. $2.4 \times 10^{-4}M$
4. $4.8 \times 10^{-4}M$

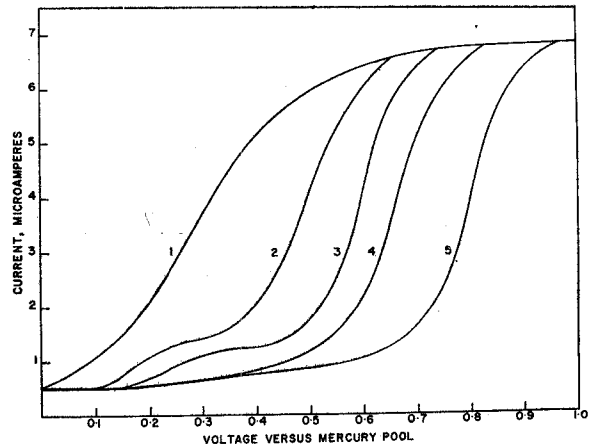

Figure 2. Effect of pH on apparent half-wave potential

(See Table III for buffers)

1. pH 0.12
2. pH 1.10
3. pH 1.97
4. pH 3.00
5. pH 3.96

Diffusion Current Dependence on Temperature. Polarograms of $1 \times 10^{-3}M$ α-methyl-DL-cystine using $1.2 \times 10^{-4}M$ thymol were run in the range of 0° to 45° C. A linear relationship between temperature and total diffusion current occurred (Table II).

Effect of pH on Apparent Half-Wave Potential. Solutions of α-methyl-DL-cystine ($1 \times 10^{-3}M$) were prepared using media of various pH levels (*3*) with $1.2 \times 10^{-4}M$ thymol. The solutions were analyzed at 21.1° C. and the apparent half-wave potential was determined (Table III).

The polarograms had different shapes (Figure 2) and the apparent half-wave potential was observed to increase negatively with increase in pH. The higher the pH, the more nearly S-shaped the polarograms became (Figure 2).

Effect of Thymol on Apparent Half-Wave Potential. The effect of thymol concentration on the shape of the polarographic wave was studied. Solutions of $1 \times 10^{-3}M$ α-methyl-DL-cystine were analyzed at different thymol concentrations and the apparent $E_{1/2}$ was determined.

The value of the apparent half-wave potential changes with thymol concentration (Table IV) because of a change in the shape of the polarographic wave (Figure 1). A well defined S-shaped curve occurred at a thymol concentration of $4.8 \times 10^{-4}M$.

Effect of Digestion on Polarographic Wave of α-Methyl-DL-Cystine. Duplicate 10-ml. samples of $1 \times 10^{-2}M$ α-methyl-DL-cystine were digested according to the method of Koch and McMeekin (*4*). A distilled water blank was treated similarly.

Polarographic analysis of the digests in 0.1*N* hydrochloric acid at 19.4° C. using $1.2 \times 10^{-4}M$ thymol revealed that the characteristic wave was absent in both the blank and the samples. This was taken as evidence that no substance present in the reagents was responsible for the wave usually obtained with α-methyl-DL-cystine.

Table III. Effect of pH on Apparent Half-Wave Potential

pH[a]	Medium Used[b]	Apparent Half-Wave Potential, Volt	
0.12	1*N* HCl	−0.318	−0.320
1.10	0.2*M* KCl + 64.5 ml. 0.2*N* HCl[c]	−0.537	−0.540
1.97	0.2*M* KCl + 10.6 ml. 0.2*N* HCl[c]	−0.579	−0.575
3.00	0.2*M* potassium acid phthalate + 20.3 ml. 0.2*N* HCl[c]	−0.663	−0.666
3.48	0.2*M* potassium acid phthalate + 6.0 ml. of 0.2*N* HCl[c]	−0.727	−0.726
3.96	0.2*M* potassium acid phthalate + 0.40 ml. 0.2*N* NaOH[c]	−0.810	−0.810

[a] Values represent final solution to be analyzed.
[b] Buffers were prepared according to Clark and Lubs (*3*).
[c] Amount of acid or base required to prepare 200 ml. of buffer solution.

Table IV. Effect of Thymol Concentration on Apparent Half-Wave Potential

Thymol Concn., Mole/Liter	Apparent $E_{1/2}$, Volt		Temperature, ° C.
2.4×10^{-5}	−0.343	−0.343	21.7
1.2×10^{-4}	−0.555	−0.555	21.9
2.4×10^{-4}	−0.652	−0.652	21.9
4.8×10^{-4}	−0.723	−0.723	18.2

Diffusion Coefficient and Reversibility of the Reaction. By substitution in the Ilkovič equation, the diffusion coefficient for a $1 \times 10^{-3}M$ solution of α-methyl-DL-cystine at 25° C., using $1.2 \times 10^{-4}M$ thymol, was found to be 9.38×10^{-6} sq. cm. per second; the diffusion current was 4.19 μa. and the capillary constant 2.253. The value of the diffusion coefficient obtained is of the same order as that reported by Kolthoff and Barnum (*6*) for cystine (5.3×10^{-6} sq. cm. per second).

The reversibility of the reaction was tested by plotting E *vs.* $\log \frac{i_D - i}{i^2}$ for $1 \times 10^{-3}M$ α-methyl-DL-cystine at 20° C. at thymol concentrations of 1.2×10^{-4} and $4.8 \times 10^{-4}M$, respectively. Straight lines were obtained for both thymol concentrations with slopes of 0.0817 and 0.0533 for thymol concentrations of 1.2×10^{-4} and $4.8 \times 10^{-4}M$, respectively. These values differ from the theoretical slope (0.0295). In this respect the results are similar to Kolthoff and Barnum (*6*), and Kalousek, Grubner, and Tochstein (*5*), who concluded that the cystine reduction is not reversible.

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