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SYNTHESIS AND POLAROGRAPHIC STUDIES
OF BETA-SUBSTITUTED PHENYLALANINE DERIVATIVES

BY

CHANDRAVADAN PATEL

A Thesis
Submitted to the Faculty of Graduate Studies through the
Department of Chemistry in Partial Fulfillment
of the Requirements for the Degree of
Master of Science at the
University of Windsor

Windsor, Ontario

1969

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ABSTRACT

N-benzoyl-threo- β -p-substituted cystine ethyl esters have been prepared. These phenyl-, p-chlorophenyl-, p-methoxyphenyl-, and p-nitrophenylcystine ethyl esters were prepared from the corresponding cysteine derivatives by oxidation. The substituted cysteine derivatives were synthesized from the corresponding N-benzoyl-S-dichloro-threo- β -p-substituted phenylcystine ethyl esters which were synthesized via the condensation of dichlorothioacetic acid and the corresponding ethyl cinnamates. Substituted cinnamates were prepared from the corresponding azlactones which in turn were synthesized from para-substituted benzaldehydes and hippuric acid.

A polarographic method was developed for the determination of N-benzoyl-threo- β -p-substituted cystine ethyl esters. An attempt was made to find a correlation between the half-wave potential and the substituent constant (Taft constant σ^*).

Ultraviolet spectra were measured for the α -substituted cystines and the N-benzoyl-threo- β -p-substituted cystine ethyl esters in appropriate solvents. A correlation between the wave length and half-wave potential was investigated.

ACKNOWLEDGEMENTS

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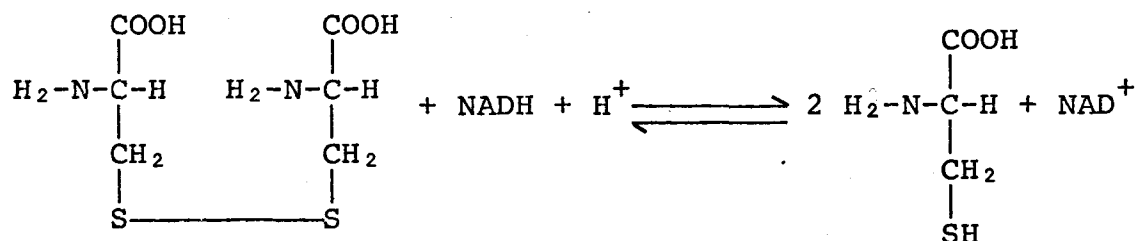
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CHAPTER I
INTRODUCTION

Polarographic studies have been carried out (1) in this laboratory on alpha-substituted cystines in aqueous media. Enzymatic reduction rates of cystine and alpha-substituted cystines have also been studied (2). Cystine reductase (EC 1.6.4.1) has been utilized in these studies.

The overall enzymatic reduction could be illustrated as:



In the literature, it has been reported that the lack of alpha-hydrogen atom tends to make the amino acid inert in many enzymatic reactions. In the case of alpha-substituted cystines as shown in Table I, the alpha-hydrogen atom is not necessary for this enzymatic reduction. However, there is no pronounced correlation between the structure and the ease of reduction of these alpha-substituted cystines.

Table II illustrates the polarographic half-wave potential and the pH values at the first and second inflection points of these alpha-substituted cystines as determined by Thibert (1). Taft values (ρ^*) have been added to the original table to establish whether one can

TABLE I

ENZYMATIC VERSUS POLAROGRAPHIC REDUCTION OF THE ALPHA-SUBSTITUTED CYSTINES

Legend

Initial velocities and first order rate constants determined using 1×10^{-3} M L-cystine, DL-cystine, α -substituted cystine in 0.025M phosphate buffer (pH 6.2). First order rate constants were calculated from the first order rate equation.

TABLE I

ENZYMATIC VERSUS POLAROGRAPHIC REDUCTION OF THE ALPHA-SUBSTITUTED CYSTINES*

Amino Acid	v_o ($\mu\text{M min}^{-1}$)	k (min^{-1})	Half-Wave Potential (volt)
L-Cystine	0.415	415	-0.536
α -Methyl	0.350	350	-0.514
DL-Cystine	0.346	346	----
α -n-Propyl	0.345	345	-0.186
α -n-Butyl	0.318	318	-0.202
α -Phenyl	0.264	264	-0.184
α -Ethyl	0.190	190	-0.249
α -Isopropyl	0.167	167	-0.370

* As shown in reference (2)

TABLE II
 PROPERTIES OF SOME AMINO ACIDS AND σ^* VALUES*

Amino Acid Cystine	Half-Wave Potential (V)	pH at first Inflection Point	pH at second Inflection Point	σ^*
L-Cystine	-0.536	10.25	8.68	+0.49
α -Methyl-DL-	-0.514	10.44	9.08	0.00
α -Ethyl-DL-	-0.249	10.33	8.62	-0.10
α -n-Propyl-DL-	-0.186	10.32	8.63	-0.11
α -n-Isopropyl-DL-	-0.370	10.38	8.67	-0.19
α -n-Butyl-DL-	-0.202	10.48	8.78	-0.13
α -Phenyl-DL-	-0.184	9.49	8.19	+0.60

* These values were obtained from reference (1)

TABLE III
 RESONANCE EFFECTS OF GROUPS*

Substituent Group	Inductive Effect	Resonance Effect	Shift of $E_{1/2}$ Towards
H	-	-	-
OCH ₃	-I	+R	positive
Cl	-I	+R	intermediate
NO ₂	-I	-R	negative

* These values were obtained from reference (3)

find any linear correlation between the structure and the half-wave potential. The σ^* values are dependent on the kind of substituent but are independent of the nature of the reactive group (-S-S-), the type of reaction, and the reaction condition. These values do not take into account the mesomeric interaction between the reactive group and the substituent (which in this case is separated by two methylene groups). It indicates the direct polar interaction between the substituent and the polarographically active group (-S-S-).

Since no linear correlation could be established between the half-wave potential and the σ^* values, one can predict that there is no polar effect due to the substituents in the alpha-position of alpha-substituted cystines. It is possible that there might be a steric interaction instead of the polar interaction. Therefore, one should use the following relationship (4):

$$E_{1/2} = P + M_{\pi} + s$$

Where P = change in the polar activation energy increment

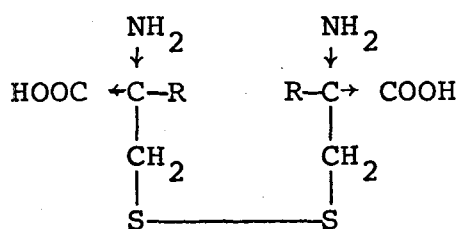
M_{π} = change in the mesomeric energy increment

s = change in the total steric energy increment

P, s and M_{π} are often independent variables.

In order to understand and differentiate between all these effects, a proper choice of selection rules for a given series of reactions have to be established. Since

steric effects are non-additive, it seems probable that no general relationship or simple correlation between the steric effect and the substituent may be found in this series compared to some other series reported in the literature (4). The unavailability of the special substituent constants makes it difficult to choose the proper kind of values. Sometimes it is possible to make an estimate based on the number of atoms between the atom carrying the substituent and that carrying the electroactive group. Normally one has to depend on a trial-and-error approach. In case of alpha-substituted cystines, both polar and steric



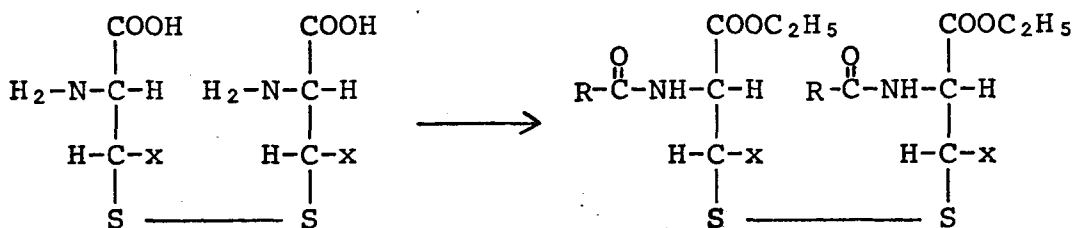
effects might be of importance since the effective carboxylic and amino group had not been taken into consideration. In such a case one could make use of the following relationship:

$$E_{1/2} = \frac{\sigma^* \text{ carboxylic} + \sigma^* \text{ amino} + \sigma^*}{3}$$

Similar calculations have been cited (4) where the correlation was carried out by the use of $(\sigma_{-m} + \sigma_{-p})/2$ values.

In order to understand the effect of substituents and their relationship to the reduction of cystine polarographically, it was thought feasible to move the substituents

from the alpha position to the beta position. In this way, a pronounced polar effect if any, could be established at the carbon atom next to the electro-active group.



Furthermore in order to understand the true polar effect on the reduction of beta-p-substituted phenylcystine polarographically, the free carboxylic- and the amino-groups were blocked in order to minimize any effects produced by hydrogen bonding, (since it is known that hydrogen bonding tends to shift $E_{1/2}$ towards a more positive potential). In order to differentiate between the various contributions by the substituents, a proper selection of the substituents had to be made according to the following rules:

- 1) It should have practically negligible steric and mesomeric effect on the reactive group.
- 2) The substituent effects should only be polar, so that the following condition could be fulfilled.

$$E_{1/2} = P \quad \text{where } P = \text{polar effect}$$

The effect of a substituent is expressed relative to that of hydrogen. An electrophilic group is more negative

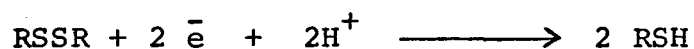
than hydrogen [-I (inductive) and -R (resonance)] while a nucleophilic group is more positive (+I and +R). Keeping these rules in mind, the following substituents were chosen for the synthesis of N-benzoyl-threo- β -p-substituted phenyl-cystine ethyl esters which were oxidized to the cystine derivatives by iodine in dioxane. Table III shows the groups and their inductive and resonance effects, as well as the type of shift one can predict for the half-wave potentials. The substitution of the group was in the para position on the benzene ring, since in the absence of other effects, the reduction of benzene derivatives with a reducible group in the side chain and a -R substituent effect, the reduction occurs at a more positive potential than at any other position of the substituent (ortho, meta). In cases where the p-substituted benzene is attached directly to the electro-active group (-S-S) as in various alkyl and diphenyl disulphides (5,6) as reported by Hall, Karchmer, and Walker, the effect of the substituents is found to be very pronounced and a linear correlation between half-wave potential and Taft's value has been established. In these cases it has proved useful to use σ^- values which were determined from the reactions of para-substituted phenols and anilines (7-9). One can then use the following equation (4):

$$E_{1/2} = \rho\pi, R \sigma^-$$

where $\rho\pi, R$ = reaction constant expressed in volts
 (expresses the susceptibility of the
 reduction process to the polar effects
 of substituents).

For reaction series of the type X-Y-R, where the reactive group R undergoes electro-reduction under certain conditions and X is the substituent which is not directly involved in the reaction, Zuman (4) has demonstrated that in such series of compounds, polar, mesomeric and steric effects can often be differentiated, and that an accurate comparison of polarographic half-wave potentials is possible.

Brdicka, Roncato, Kolthoff and Barnum (10-13) have studied cystine polarographically in aqueous media. They have established that the half-wave potential in this media is pH dependent and have shown the quantitative relationship between diffusion current (wave-height) and the concentration of the cystine. The rate-determining step of the electrochemical reduction is believed to be the cleavage of the (-S-S-) bond in a two-electron step as shown here:



The equation of the polarographic wave then is given as follows (14):

$$E_{D.M.E} = E^1 + 0.0295 \log (i_d - i)/i^2$$

where $E^1 = E^0 - 0.0591 \text{ pH}$ and predicts that a plot of $E_{D.M.E}$ vs $\log i_d - i/i^2$ should be linear with a slope of 29.5 mV. Furthermore, the above equation predicts that $E_{1/2} = E^1 - 0.0295 \log i_d/2$

Investigation of the p-substituted cystine derivatives and their electrochemical behaviour has been carried out using conventional polarography in non-aqueous media.

Other studies including oxidation-reduction reactions of cysteine-cystine as well as chelate formation with copper salts have been reported in literature (15). Chelates of cysteine have been used to afford some protection against the effect of ionizing radiation on the animal (rat) cell (16, 17). All these substituted cysteines and cystines (α and β) could be further investigated along this line. Thibert and co-workers used N-bromosuccinimide in the simultaneous determination of cysteine-cystine in enzymatic reactions. This quantitative method which uses Bordeaux Red as an indicator could be used to determine the beta-substituted cysteines and cystines (18, 19).

The relation between the half-wave potentials and the absorption spectra of polycyclic aromatic hydrocarbons has been studied by Bergman (20). An attempt was made in our studies to correlate the half-wave potentials of the β-phenyl-cystine derivatives with the absorption band at $\lambda_{\text{max}} 204$ which is characteristic of disulphide absorption (21).

CHAPTER II

EXPERIMENTAL

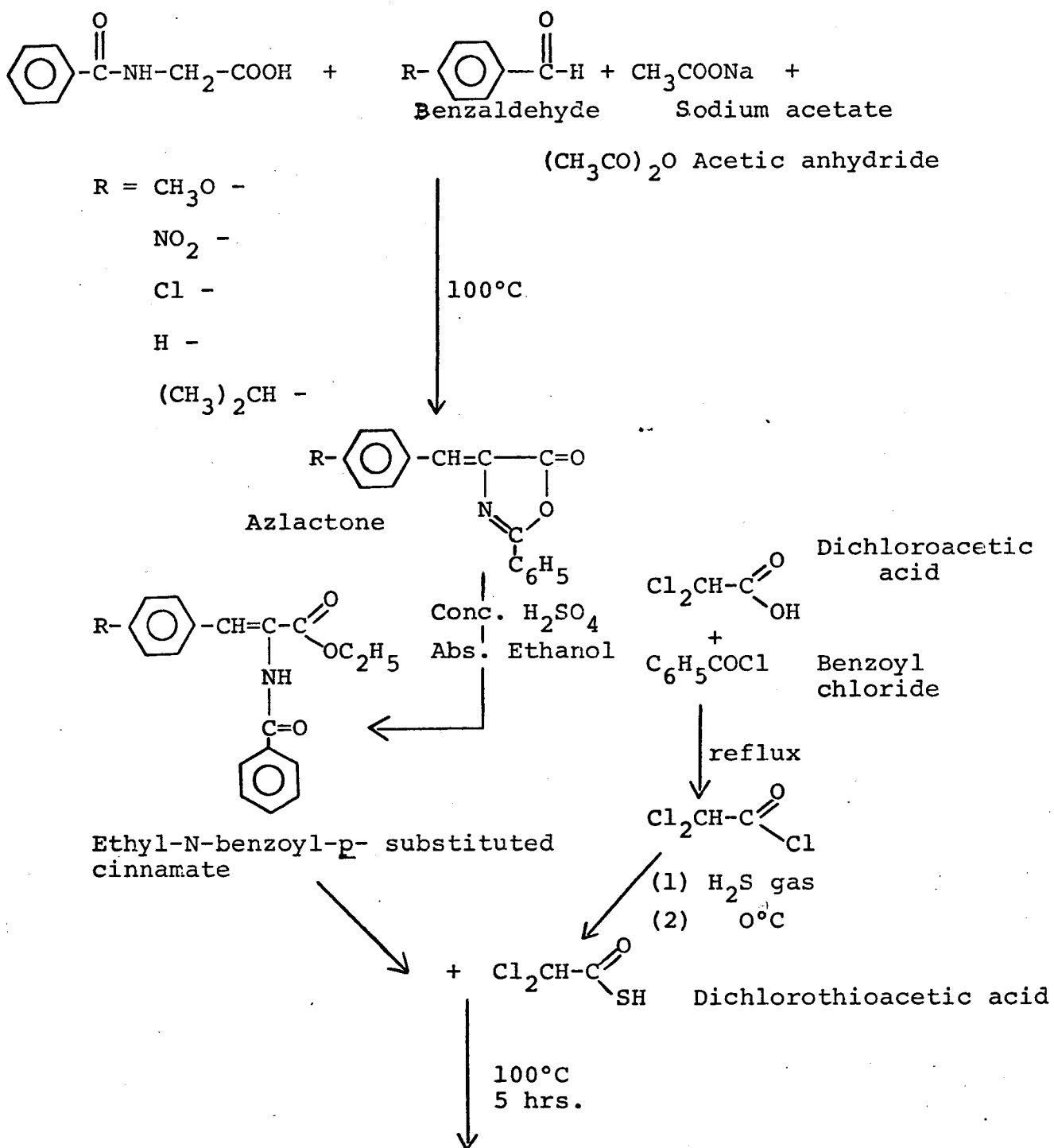
A. The Synthesis of Beta-Substituted Phenylcystine Derivatives

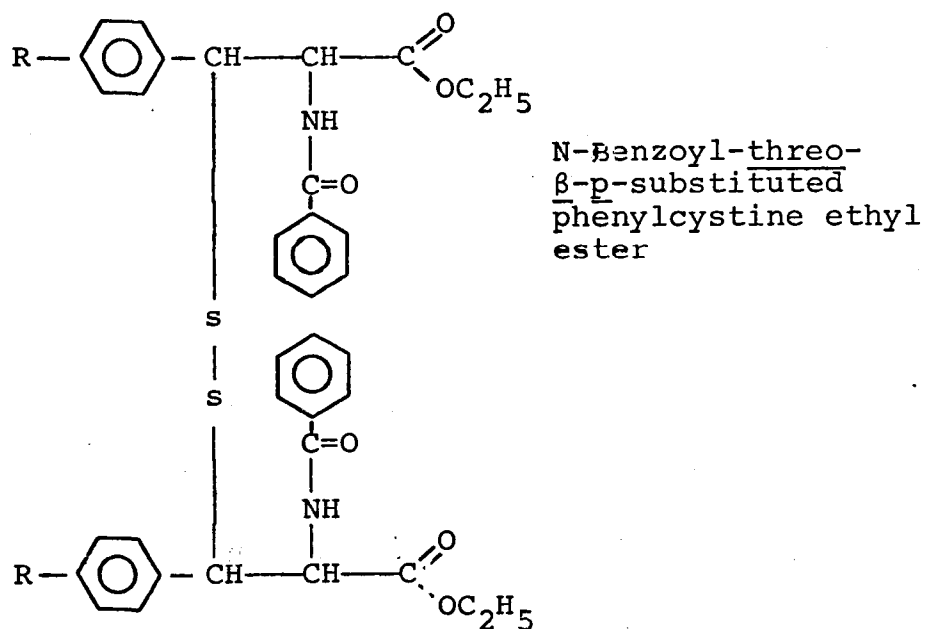
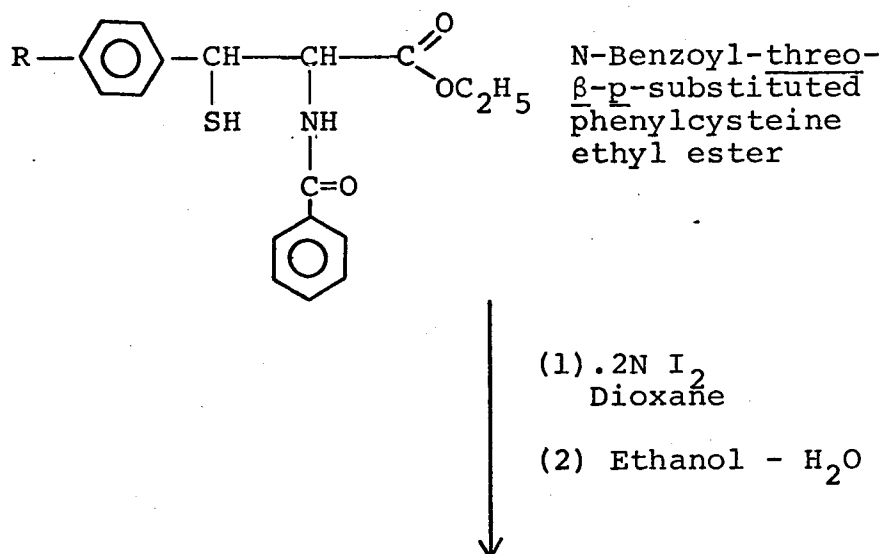
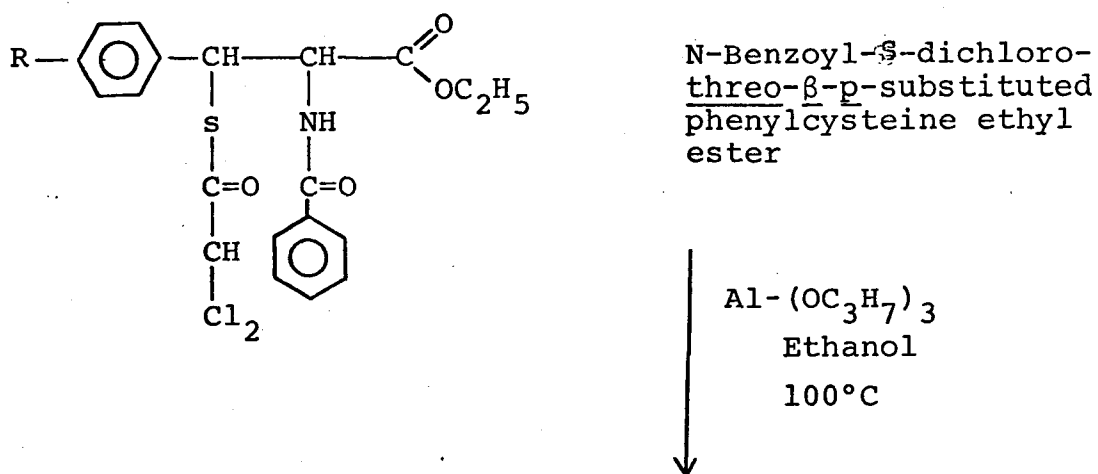
The following substituted amino acid derivatives were synthesized: N-benzoyl-threo- β -phenylcystine ethyl ester, N-benzoyl-threo- β -p-methoxyphenylcystine ethyl ester, N-benzoyl-threo- β -p-chlorophenylcystine ethyl ester, and N-benzoyl-threo- β -p-nitrophenylcystine ethyl ester. The syntheses and configuration of the β -phenylcysteines have been previously reported (23,24). As one of the typical examples of the series, Figure II shows in detail the synthesis of N-benzoyl-threo- β -phenylcystine ethyl ester. Most of the intermediates and all of the four final products were synthesized for the first time in this laboratory.

Elemental analyses were carried out by the Schwarzkopf Microanalytical Laboratory, 56-19 37th Avenue, Woodside 77, New York. Infrared spectra were run on each intermediate and the final compound and the specific bands identified in our laboratories. Melting points reported here are uncorrected. A Fisher-Jones melting point apparatus and a Beckman IR-10 spectrophotometer were used in these studies.

FIGURE 1

SYNTHESIS OF BETA-SUBSTITUTED PHENYLALANINE DERIVATIVES





Azlactone (oxazolones)

The procedure of Erlenmeyer et al. (25-29) was employed to prepare these substituted azlactones. In the synthesis of 2-phenyl-4-benzylidene oxazolone, hippuric acid (54.2 g; 0.33 mole); sodium acetate anhydrous (24.6 g; 0.33 mole); benzaldehyde (31.8 ml; 0.33 mole) and acetic anhydride (85.1 ml; 0.83 mole) were shaken at intervals of 2 minutes for the first fifteen minutes on a steam bath until a clear yellow liquid was obtained. The mixture was further heated for one hour during which time a solid product was obtained. The mixture was cooled, filtered with suction, washed with alcohol to remove excess benzaldehyde and recrystallized from benzene. The melting point of the product (pale yellow needles) was 164-166°C (yield 75%). The reported mp is 165-166°C.

In a similar manner the following substituted oxazolones, 4-p-nitrobenzylidene- (mp 235-237°C dec; yield 67%), 4-p-methoxybenzylidene- (mp 155-156°C; lit. mp 156.5°C; yield 42%), 4-p-chlorobenzylidene- (mp 195-196°C, yield 55%) and 4-p-isopropylbenzylidene- (mp 119-121°C; lit. mp 121°C; yield 34%) were prepared using p-nitrobenzaldehyde, anisaldehyde, p-chlorobenzaldehyde and p-isopropylbenzaldehyde, respectively. Infrared spectra of the compounds in KBr pellets were recorded.

Ethyl-N-benzoyl-p-substituted cinnamate

A route previously found by Erlenmeyer et al. (25-29) was utilized in the preparation of these esters. The preparation of ethyl- α -benzamidocinnamate will illustrate a typical example of these syntheses.

A suspension of 2-phenyl-4-benzylidene oxazolone (12 g; 0.05 mole) was refluxed with a mixture of absolute ethanol (75 ml; 2.1 mole) and concentrated sulphuric acid (5 ml) for 3-4 hours until a clear solution was obtained. Excess ethanol was evaporated on a water-bath and the white residue was washed with water until the filtrate was free from any traces of sulphuric acid. The crude product was recrystallized from ethanol. The mp was 148-149°C; (yield 85%). The reported value is 149°C.

Using the above procedure the following compounds: ethyl- α -benzamido-p-methoxycinnamate (mp 146-147°C; yield 91.6%); ethyl- α -benzamido-p-chlorocinnamate (mp 149-150°C; yield 73.3%); ethyl- α -benzamido-p-nitrocinnamate (mp 165-166°C; yield 80%; lit. mp 165-166°C); and ethyl- α -benzamido-p-isopropylcinnamate (mp 161-163°C; yield 92%; lit. mp 164°C) were prepared. Infrared spectra were run on each of these compounds using KBr pellets.

N-Benzoyl-S-dichloroacetyl-threo- β -p-substituted phenyl-cysteine ethyl esters

The method of Sicher et al. (23,24) was used for the

synthesis of these series of compounds. The preparation of N-benzoyl-S-dichloroacetyl-threo- β -p-phenylcysteine ethyl ester will serve as a typical example of these series.

A mixture of ethyl- α -benzamidocinnamate (6.8 g; 0.023 mole) and dichlorothioacetic acid (10 ml) was refluxed on a steam bath for 5 hours until a viscous reddish syrup was obtained. After cooling and addition of ether, scratching immediately induced the separation of white crystals, which after crystallization from ethanol gave a mp 128-131°C (yield 40%; lit. mp 128-131°C).

In the same manner the following analogous p-methoxy- (mp 108-109°C; yield 31%); p-chloro- (mp 133-134°C; yield 30.9%); p-isopropyl- (mp 146°C; yield 0.6%) and p-nitro- (mp 142-143°C; yield 38%; lit. mp 142°C) esters were prepared. In the case of the p-isopropyl- derivative, crystallization took as long as a week. A good yield of p-isopropyl adduct was not obtained and, therefore, no further work was done on this compound. In the case of p-nitro adduct, it was necessary to remove the excess dichlorothioacetic acid prior to the addition of ether.

N-Benzoyl-threo- β -p-substituted phenylcysteine ethyl esters

The reduction of the N-benzoyl-S-dichloroacetyl-threo- β -p-substituted phenylcysteine ethyl esters was accomplished using the Meerwein-Ponndorf-Verley method (30).

N-Benzoyl-S-dichloroacetyl-threo- β -phenylcysteine ethyl ester (2.2 g; 0.005 mole) and powdered aluminium isopropoxide (1 g; 0.005 mole) in ethanol (50 ml) were refluxed for four hours in a 100-ml, round-bottomed flask equipped with a condenser. Excess ethanol was evaporated and the dry residue was treated with 15% tartaric acid solution (100 ml). The mixture was filtered and the residue dissolved in ether. On evaporation of ether, a white solid was obtained which was crystallized from isopropanol (mp 125-126°C; yield 70%; lit. mp 125-126°C).

Similarly, p-methoxy-, p-chloro-, and p-nitrophenylcysteine ethyl esters were prepared. In the case of the p-nitro-compound, a residue was formed during the refluxing period, which was filtered before evaporation. The yield, analysis and the mp of the white residue for the N-benzoyl- β -p-substituted phenylcysteine ethyl esters were as follows:

p-methoxy- (yield 41.6%; mp 139-140°C)

Anal. calcd. for $C_{18}H_{19}O_3NS$; C, 62.42; H, 6.04; N, 4.66
S, 9.40.

Found: C, 62.49; H, 6.02; N, 4.02; S, 9.17.

p-chloro- (yield 37%; mp 147-148°C)

Anal. calcd. for $C_{18}H_{18}O_3NSCl$: C, 59.16; H, 5.25; N, 4.04;
S, 8.87; Cl, 9.78.

Found: C, 59.40; H, 4.95; N, 3.85; S, 8.81; Cl 9.78.

p-nitro- (yield 75%; mp 134-135°C)

Found: C, 50.38; H, 4.94. These analysis (of C and H) correspond to β -p-nitrophenylcysteine ethyl ester.

Anal. calc. for $C_{11}H_{14}O_4N_2S$: C, 48.88; H, 5.18.

Dichloroacetyl chloride

The method employed by Brown (31) for the preparation of chloroacetyl chloride was used. Yield obtained was about 65% with a boiling point of 107°C, which agrees well with the literature boiling point 107-108°C.

Dichlorothioacetic acid

The conversion of dichloroacetyl chloride to dichlorothioacetic acid has been reported by Cunneen (32). A rapid stream of dry hydrogen sulphide gas was passed through a well-cooled (0°C) solution of dichloroacetyl chloride (500 g; 3.76 mole) and powdered dry aluminium chloride (10 g) until the evolution of hydrogen chloride ceased (8-10 hrs). Distillation of the reaction mixture yielded dichlorothioacetic acid as a pale green liquid (bp 56°C/16 mm; yield 60.9%; lit. bp 56°C/16mm).

N-benzoyl- β -p-substituted phenylcysteine ethyl esters

The oxidation of cysteine derivatives to disulphides was accomplished by use of 0.2N iodine solution in dioxane. The excess iodine was then removed by treatment with ethanol-water. The following synthesis of N-benzoyl-threo- β -p-phenylcysteine ethyl ester will serve to illustrate a typical case. A cysteine derivative (250 mg) was dissolved in 40 ml

of dioxane. This clear solution was titrated with 0.2N iodine solution until an excess of iodine was indicated by starch paper. The solution was evaporated to dryness and the residue treated with 5-10 ml of an ethanol-water mixture. The white precipitate formed was filtered and washed with a small amount of ethanol and recrystallized with ethanol or ethanol-water. The yield was nearly quantitative, although a considerable loss resulted from washing and recrystallization. (Yield 150 mg; mp 135°-138°C). No attempt was made to recover the lost product from the solvent. Infrared spectra indicated the disappearance of the SH band.

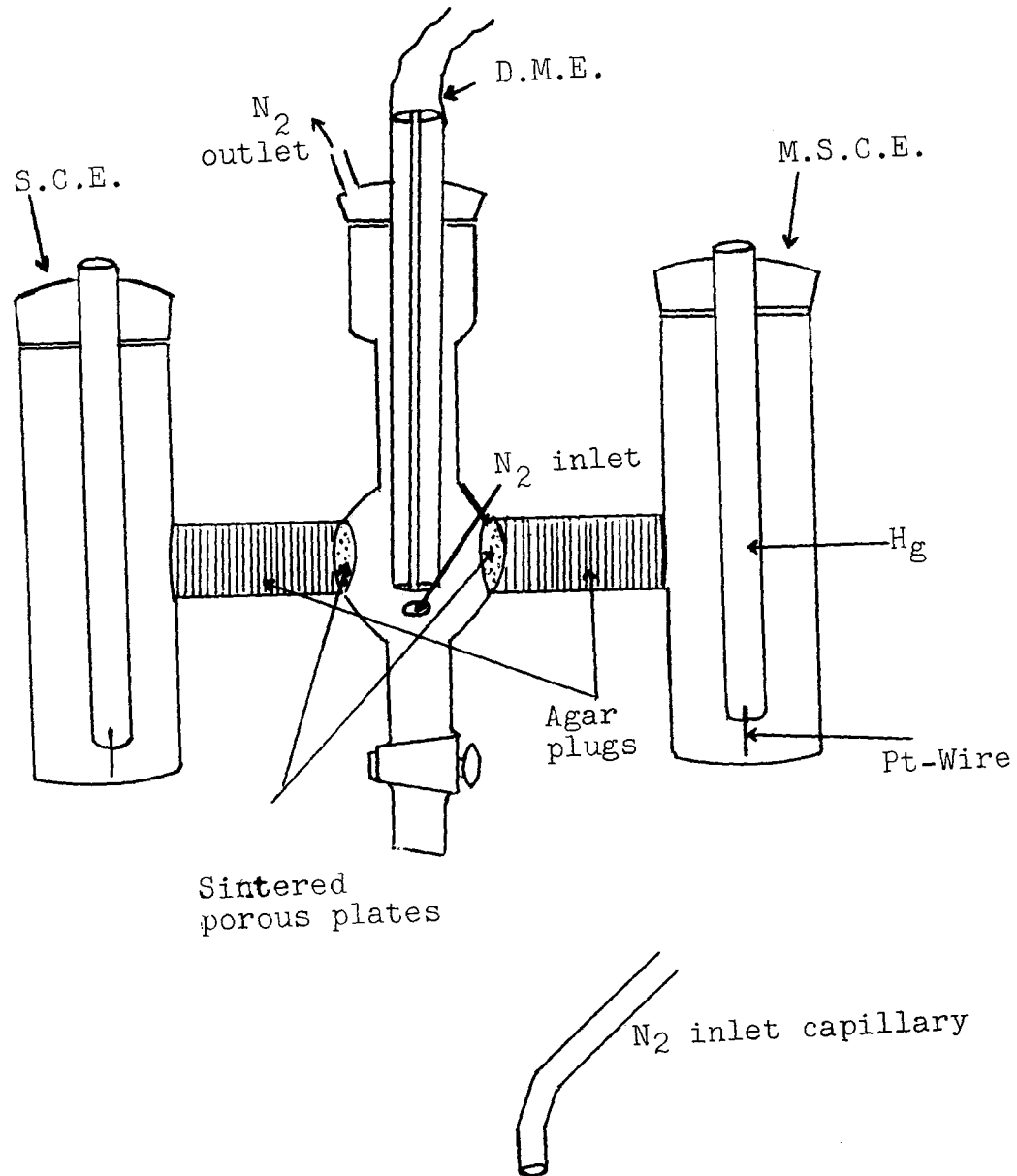
The above method was used in the synthesis of the p-methoxy- (mp 153-155°C); p-chloro- (mp 198-200°C); and p-nitro- (mp 230-232°C) derivatives. The yields obtained ranged from 40-75%.

B. Polarographic Measurements of Beta-Substituted Phenylcystine Derivatives

Materials and Methods

The Sargent (Sargent-Welch Scientific Company, 7300 North Linder Avenue, Skokie, Illinois) Model XVI Polarograph and Model A I.R. compensator were used for this study. It was found necessary to develop and make use of a special H-type cell as illustrated in Figure 2, for the non-aqueous polarographic studies (33). The need for a reference electrode that would not involve too much contact of water with anhydrous methanol, led us to make a calomel electrode in methanol. A paste of calomel and mercury was placed on a mercury pool and covered with methanol previously saturated with potassium chloride at room temperature (25°C). The system was allowed to reach equilibrium by letting it stand for a couple of days. This cell was measured against an aqueous S.C.E. Both reference electrodes were closed with ground glass joints to prevent evaporation. The resistance of the dropping-mercury electrode (D.M.E.) with respect to the saturated calomel reference electrode (S.C.E) (2500 Ω) and (D.M.E.) with respect to the methanol-saturated calomel electrode (M.S.C.E) (5700 Ω) was measured using a conductivity bridge. Both reference cells were connected to D.M.E. by a side arm containing an agar plug saturated with KCl. All

FIGURE 2
THE CELL



potential measurements were made with respect to an aqueous S.C.E. A Fluke Model 825 A Differential Voltmeter was used to measure the potential against the M.S.C.E.

All electrochemical experiments were carried out at $25 \pm 0.1^\circ\text{C}$. The electrolyte used was 0.1M CH_3COONa in anhydrous methanol. The apparent pH of the solution was 9.7. The solutions were deaerated with nitrogen. The removal of residual oxygen from the nitrogen was accomplished by passing it through a train consisting of (in order) a bubbling tower filled with 1:1 NH_4OH and H_2O (containing a few pieces of copper turnings and saturated with NH_4Cl), a tower filled with concentrated sulfuric acid; an empty trap and two towers containing electrolyte solution, the second having been immersed in water bath of $25^\circ \pm 0.10^\circ\text{C}$.

No damping was used in recording any of the polarograms. Methanol was purified by reflux and distillation from a magnesium-iodine solution at a high reflux ratio (34).

The characteristics of the capillary used were:

$m = 7.53 \text{ mg sec}^{-1}$; $t = 5.6 \text{ sec}$; $m^{2/3}t^{1/6} = 1.623$
 $\text{mg}^{2/3} \text{ sec}^{-1/2}$. The height of the mercury column was 53.5 cm.

Stock solutions of beta-substituted phenylcystine derivatives were prepared by dilution of accurately weighed portions of the appropriate substituted phenylcystines. Solutions for electrochemical experiments were prepared

using aliquots of the stock solution and dilution to the proper volume with stock electrolyte solution. No maximum suppressor was used in these studies. The diluted solutions were transferred to the polarographic cell, and deaerated for 15-30 min. prior to recording of polarograms at $25 \pm 0.1^{\circ}\text{C}$.

No correction for junction potentials have been made in this study.

CHAPTER III

RESULTS

Polarographic Measurements of Some Beta-Substituted Phenylcystine Derivatives

Effect of Concentration on Diffusion Current

Polarograms for N-benzoyl-threo-β-p-substituted phenylcystine ethyl ester in methanol with 0.1M sodium acetate as supporting electrolyte gave only one wave with the exception of the p-nitro derivative. In this case two waves were obtained. The first wave represented the reduction of the disulphide, while the second wave that of the nitro group. A linear relationship of the diffusion current to the concentration was obtained (Table IV and Figure 3). No prewave was observed as reported by Kalousek, et al. for the case of the polarographic reduction of cystines and α-substituted cystines as shown by Thibert and Walton in aqueous medium (1, 35).

Calculations of diffusion current was done by measuring the height, *h*, of the polarographic wave in millimeters (Figure 4) and substituting in the following equations:

$$i_d = \text{Sens.} \times h \quad (1)$$

Where i_d = the diffusion current in μa

Sens = the sensitivity in $\mu\text{a}/\text{mm}$

h = the wave height in mm

e.g. Sens = 0.020 μa per mm

h = 151 mm

TABLE IV
EFFECT OF CONCENTRATION ON DIFFUSION CURRENT

Amino Acid ** Concentration (Mole/Liter)	Diffusion Current (μ A)			
	H	Methoxy	Chloro	Nitro*
1×10^{-4}	.541	.587	.491	1.192
8×10^{-5}	.448	.487	.402	1.078
5×10^{-5}	.310	.304	.281	.848
3×10^{-5}	-	-	-	.626
2×10^{-5}	-	.141	.117	
1×10^{-5}	.070	-	-	-

All the values are an average of three determinations.

*Diffusion current measured from the first wave

** Amino acid - appropriate N-benzoyl-threo- β -p-substituted phenyl-cystine ethyl ester.

FIGURE 3

EFFECT OF CONCENTRATION ON DIFFUSION CURRENT

Legend

Data for these plots are taken from current-voltage curves uncorrected for residual current.

Plots given are for (1) p-chloro-, (2) H-, (3) p-methoxy, (4) p-nitro-substituted-β-phenylcystine derivatives.

FIGURE 3
EFFECT OF CONCENTRATION ON DIFFUSION CURRENT

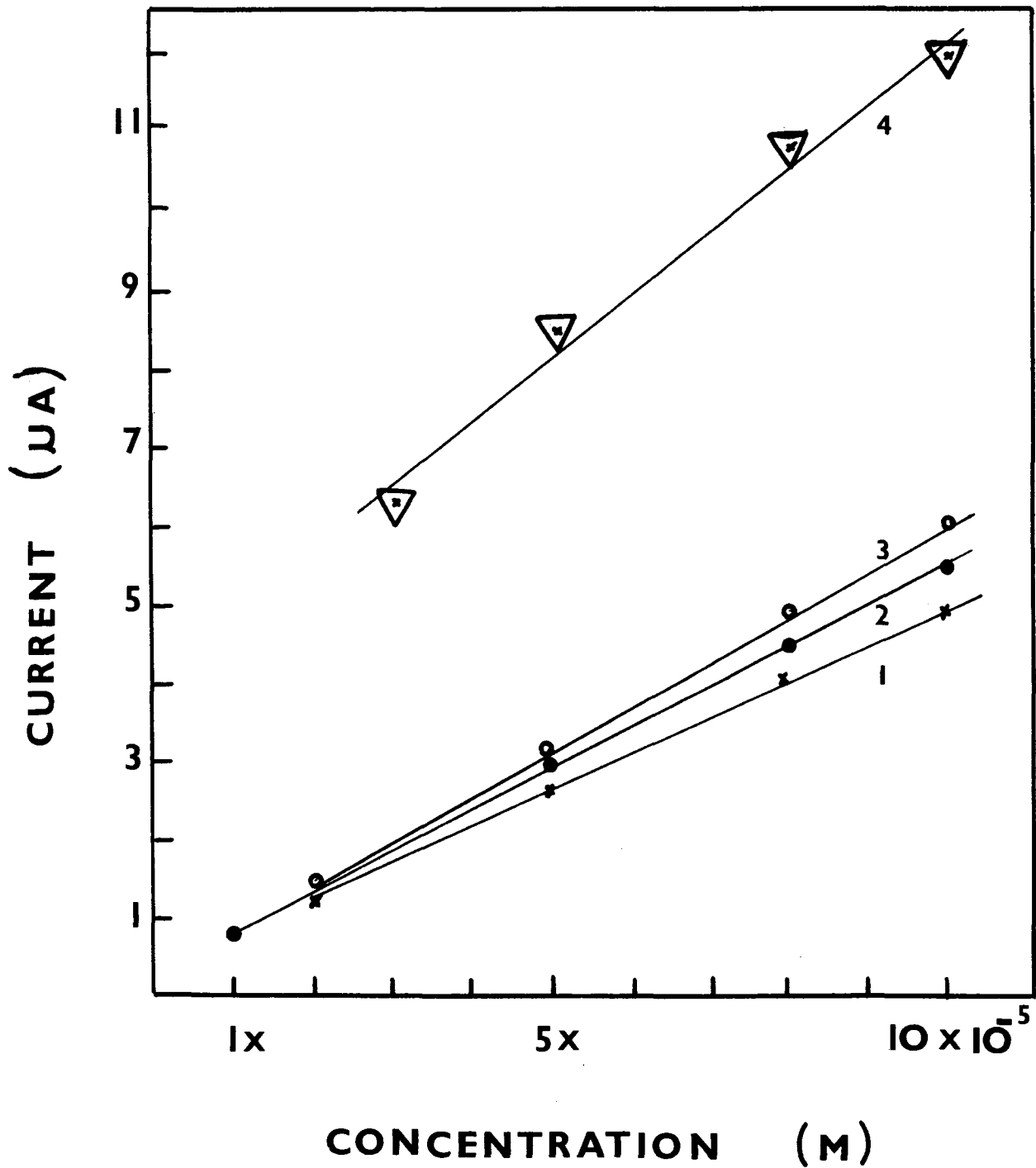
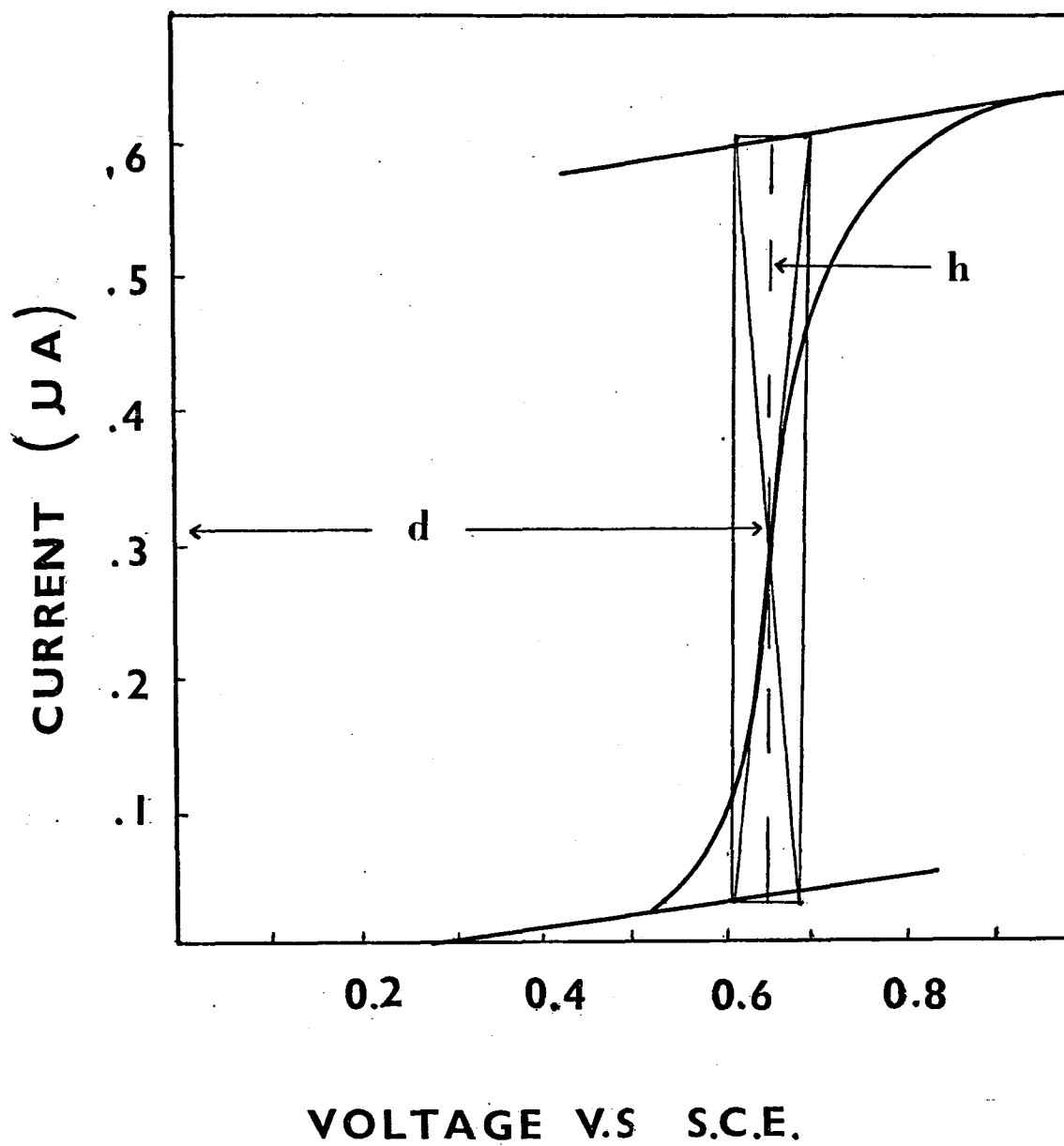


FIGURE 4
TYPICAL POLAROGRAM

Legend

A typical polarogram of N-benzoyl-threo- β -p substituted phenylcystine ethyl ester in 0.1M sodium acetate-methanol. It shows the method by which the height, h, (diffusion current) and the apparent $E_{1/2}$ are measured.

FIGURE 4
TYPICAL POLAROGRAPH



$$\begin{aligned}\text{Therefore: } i_d &= 0.0020 \times 151 \\ &= 0.302 \mu\text{a.}\end{aligned}$$

The apparent half-wave potentials were calculated by measuring the distance in inches (d) from the beginning of the polarogram to the middle of the diffusion current wave curve (36) as shown in Figure 4.

These values were then substituted in equation 2:

$$E_{1/2} \text{ app.} = (-1/10) \times d \quad (2)$$

Where $E_{1/2} \text{ app.}$ = the apparent half-wave potential

$1/10$ = a const. for polarograph (since a polarogram is 10 inches long and the span used was 1 volt, therefore 1 inch represents 0.1 volt on the polarogram from the start (0 volt) of the span). The negative sign represents the negatively increasing voltage.

d = the distance in inches from the start

Therefore: if the polarogram was started at 0 volt and the d = 6.38 inches

$$\begin{aligned}\text{The } E_{1/2} \text{ app} &= -.1 \times 6.38 \\ &= -0.638 \text{ volt}\end{aligned}$$

The true half wave potentials were calculated according to the method of Taylor and Smith (37). All half-wave potential measurements were made with a manual arrangement, using a Fluke Model 825 A Differential voltmeter. The voltage was applied to the cell by means of the slide wire of the Sargent Model XVI polarograph. The potential

of the dropping-mercury electrode (D.M.E.) with respect to the saturated calomel reference electrode (S.C.E) was measured with a precision Fluke differential voltmeter. The current and voltage were measured at a point about 0.1 volt or more, preceding the apparent half-wave potential to establish a zero point for the wave, and at appropriate voltage increments (of .05 V) up to and including the diffusion current plateau. Similar measurements were carried out on the supporting electrolyte solution alone. This value gave the residual current, which was then subtracted from the diffusion current readings (i). The diffusion current i_d was then calculated. From these values of the current, $\log \frac{(i_d - i)}{i}$ values were calculated and plotted against the measured voltage. The value of E at $\log \frac{(i_d - i)}{i} = 0$ corresponds to the true half-wave potential, when the IR compensator is used in the circuit to correct for the iR drop.

Diffusion Coefficient and Reversibility of the Reaction

The diffusion current obtained at the D.M.E. under controlled (36) conditions is dependent on diffusion and electrolysis factors. The diffusion parameter includes temperature, viscosity, ionic mobility, the area and drop rate of the mercury. Ilkovic has developed the following equation for the polarographic diffusion current under constant conditions:

$$i_d = 607 \cdot n \cdot D^{1/2} \cdot C \cdot m^{2/3} \cdot t^{1/6} \quad (36, 44)$$

Where i_d = the average current in microamperes

n = number of faraday required per mole of electroactive species

D = the diffusion coefficient of the species in $\text{cm}^2 \text{sec}^{-1}$

C = concentration of the substance in millimoles per liter

m = the rate of flow of mercury in milligrams per second

t = drop time in seconds

Table V illustrates the diffusion coefficients of phenyl-p-methoxyphenyl-, p-chlorophenyl-, and p-nitrophenyl- β -substituted cystine derivatives calculated by the use of the Ilkovic equation.

In order to determine the number of electrons consumed in the electrode process and to demonstrate the reversibility of the system, the so-called logarithmic analysis of the current-voltage curve was carried out. The equation of the polarographic curve for a cathodic reduction may be written as (14, 44).

$$E_{D.M.E.} = E_{O.5} + \frac{0.0591}{n} \log (i_d - i) / i^2 \text{ at } 25^\circ\text{C}$$

It follows from this equation that the dependence of

TABLE V
DIFFUSION COEFFICIENT

Amino Acid Substituent Group	Diffusion Current (μ a)	Diffusion Coefficient ($\text{cm}^2 \text{sec}^{-1} \times 10^6$)
H	.276	7.84
CH ₃ O	.258	6.85
Cl	.256	6.74
NO ₂	.730	54.89

The amino (0.1M) acid concentration was 5×10^{-5} M.

Sodium acetate in methanol was used as the supporting electrolyte.

$\log (i_d - i)/i^2$ on the potential E must be a straight line with a slope of 29.5 mV. Figure 5 shows that all of the amino acids give straight lines. The slopes of these plots are given in Table VI. Table VII gives the values of E vs. $\log (i_d - i)/i^2$.

Half-Wave Potential

Corrected half-wave potentials for the amino acids, phenyl-, p-methoxyphenyl-, p-chlorophenyl- and p-nitrophenylcystine derivatives are given in Table VIII. Determination of half-wave potential was carried out after subtraction for residual current. Plots of E versus $\log (i_d - i)/i$ were drawn and the $E_{1/2}$ determined (See Figure 6). (1,44).

Ultraviolet Spectra

The wave lengths of the N-benzoyl-threo- β -p-substituted phenylcystine derivatives were measured in methanol. α -Substituted cystines were dissolved and their ultraviolet spectra were measured. Absorption data are listed in Table IX. Plots of $E_{1/2}$ versus $m\mu$ were not made since the deviation between these values were not large.

FIGURE 5
PLOT OF LOG $(i_d - i)/i^2$ VS. VOLTAGE

Legend

Data for these plots were taken from current-voltage curves corrected for residual current. The concentration of the amino acids was $t \times 10^{-5}$ M.

Plots are drawn for the N-benzoyl-threo- β -p-substituted-phenylcystine ethyl ester where (\odot) represents phenyl-, (Δ) p-methoxyphenyl-, (\times) p-chlorophenyl-, and (\bullet) p-nitrophenyl-substituents. The polarograms were run using 0.1M sodium acetate in methanol as the supporting electrolyte.

FIGURE 5
PLOT OF $\text{LOG } (i_d - i) / i^2$ VS. VOLTAGE

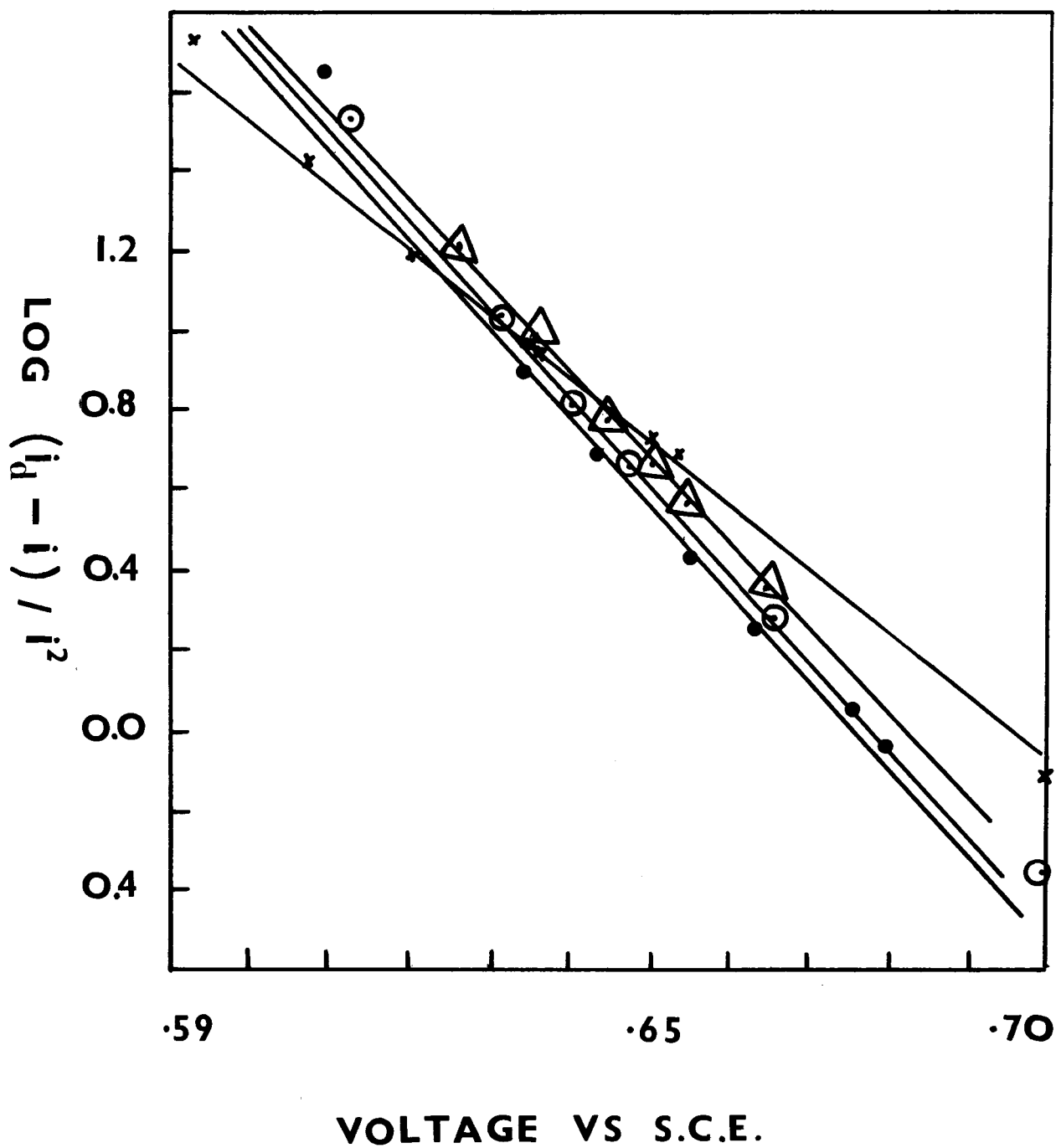


TABLE VI
SLOPE OF E VS. $\log (i_d - i)/i^2$ PLOT

Amino Acid ($5 \times 10^{-5} M$)	Slope (Volts)
N-Benzoyl- <u>threo</u> - β -phenyl- cystine ethyl ester	0.0246
N-Benzoyl- <u>threo</u> - β - <u>p</u> -methoxyphenylcystine ethyl ester	0.0208
N-Benzoyl- <u>threo</u> - β - <u>p</u> - chlorophenylcystine ethyl ester	0.0185
N-Benzoyl- <u>threo</u> - β - <u>p</u> - nitrophenylcystine ethyl ester	0.0212

TABLE VII
VALUES OF E AND $\log (i_d - i)/i^2$

Amino Acid ($5 \times 10^{-5} M$)	E (Volt)	$\log \frac{(i_d - i)}{i^2}$
N-Benzoyl- <u>threo</u> - β - phenylcystine ethyl ester	-0.613	1.539
	-0.640	0.814
	-0.665	0.258
	-0.630	1.034
	-0.700	-0.381
	-0.647	0.656
N-Benzoyl- <u>threo</u> - β - <u>p</u> -methoxyphenyl- cystine ethyl ester	-0.626	1.198
	-0.635	0.982
	-0.650	0.661
	-0.665	0.361
	-0.655	0.564
	-0.645	0.779
N-Benzoyl- <u>threo</u> - β - <u>p</u> -chlorophenylcystine ethyl ester	-0.607	1.426
	-0.620	1.193
	-0.635	0.954
	-0.650	0.722
	-0.700	-0.128
	-0.592	1.736
	-0.634	0.975
	-0.653	0.673

TABLE VII (Continued)

Amino Acid ($5 \times 10^{-5} \text{M}$)	E (Volt)	$\log \frac{(i_d - i)}{i^2}$
N-Benzoyl- <u>threo</u> - β -	-0.609	1.651
<u>p</u> -nitrophenylcystine	-0.629	1.072
ethyl ester	-0.643	0.679
	-0.663	0.256
	-0.679	-0.036
	-0.634	0.885
	-0.675	0.035
	-0.655	0.420

TABLE VIII
HALF-WAVE POTENTIAL

Amino Acid	Half-Wave Potential (Volt)
N-Benzoyl- <u>threo</u> - <u>β</u> - <u>p</u> - phenylcystine ethyl ester	-0.638
N-Benzoyl- <u>threo</u> - <u>β</u> - <u>p</u> - methoxyphenyl- cystine ethyl ester	-0.639
N-Benzoyl- <u>threo</u> - <u>β</u> - <u>p</u> - chlorophenyl- cystine ethyl ester	-0.638
N-Benzoyl- <u>threo</u> - <u>β</u> - <u>p</u> - nitrophenyl- cystine ethyl ester	-0.655

FIGURE 6

PLOT OF LOG $(i_d - i)/i$ VS. E.Legend

Data for these plots were taken from current-voltage curves. The values are all corrected for the residual current. Concentration of the amino acids used were 5×10^{-5} M using 0.1M sodium acetate in methanol as the supporting electrolyte. Plots represent phenyl- (\times), p-methoxyphenyl- (\odot), p-chlorophenyl- (\bullet), and p-nitrophenyl- (Δ) cystine derivatives.

FIGURE 6
PLOT OF $\text{LOG } (i_d - i) / i$ VS. E.

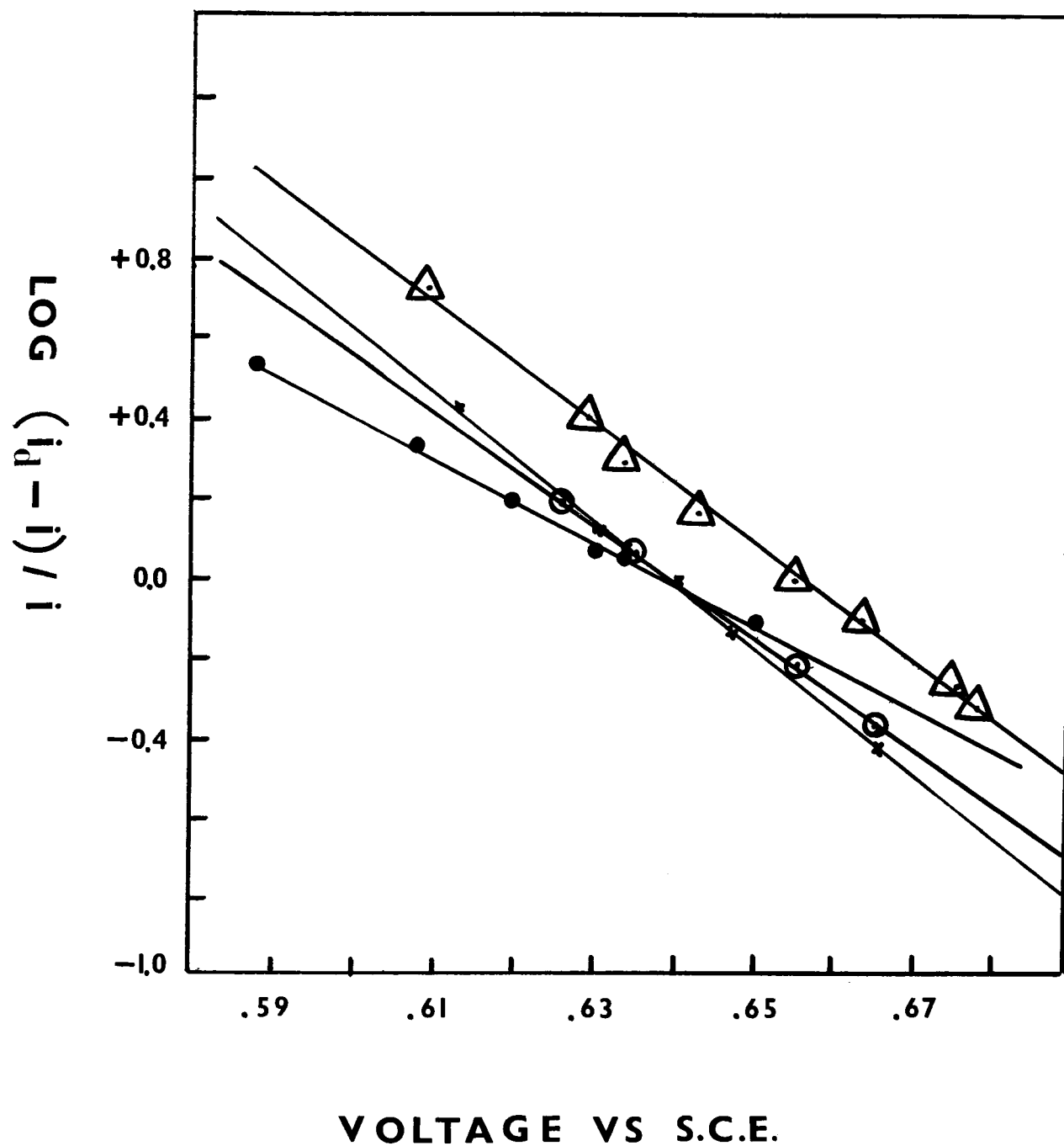


TABLE IX
 ABSORPTION CHARACTERISTICS OF SOME AMINO ACID
 AND DERIVATIVES*

Amino Acid*	λ_{\max} (m μ)	Solvent
α -Methylcystine	208	0.1N HCl
α -Ethylcystine	207	0.1N HCl
α -n-Propylcystine	205	0.1N HCl
α -Isopropylcystine	207	0.1N HCl
α -n-Butylcystine	207	0.1N HCl
α -Phenylcystine	208	0.1N HCl
β -Phenyl-	180	methanol
β -p-Methoxyphenyl-	181	methanol
β -p-Chlorophenyl-	180	methanol
β -p-Nitrophenyl-	182	methanol

* Amino acid derivatives - appropriate N-benzoyl-threo- β -p-substituted cystine ethyl ester.

CHAPTER IV

DISCUSSION

A. Synthesis of β -p-Substituted Phenylcystine Derivatives

A considerable amount of work has been carried out by Heilbron and co-workers (38-43) on the synthesis of analogues of N-benzoyl-threo- β -phenylcystine ethyl ester. Figures 7 and 8 illustrate the pathways by which they synthesized substituted phenylcysteines. A good yield of 2-mercapto thiazolone (See Figure 8), which would have been the starting material for the preparation of substituted phenylcystine derivatives, was not obtained. Both pathways gave poor yields. Among the projects rejected by Heilbron was an attempt to condense thioacetic acid with ethyl acetamidocinnamate. This was due to the low reactivity of thioacetic acid towards double bonds. Thiobenzoic acid does form an adduct but in poor yield. Sicher and co-workers (23) have demonstrated that dichlorothioacetic acid, which has a higher activity towards double bonds, forms very easily an adduct with ethyl- α -benzamidocinnamate. They have prepared substituted N-benzoyl-threo- β -phenylcystine ethyl ester via Δ^2 -oxazolines which were prepared from 1, 2-aminoalcohols.

Erlenmeyer and co-workers (25-29) have used a general

FIGURE 7

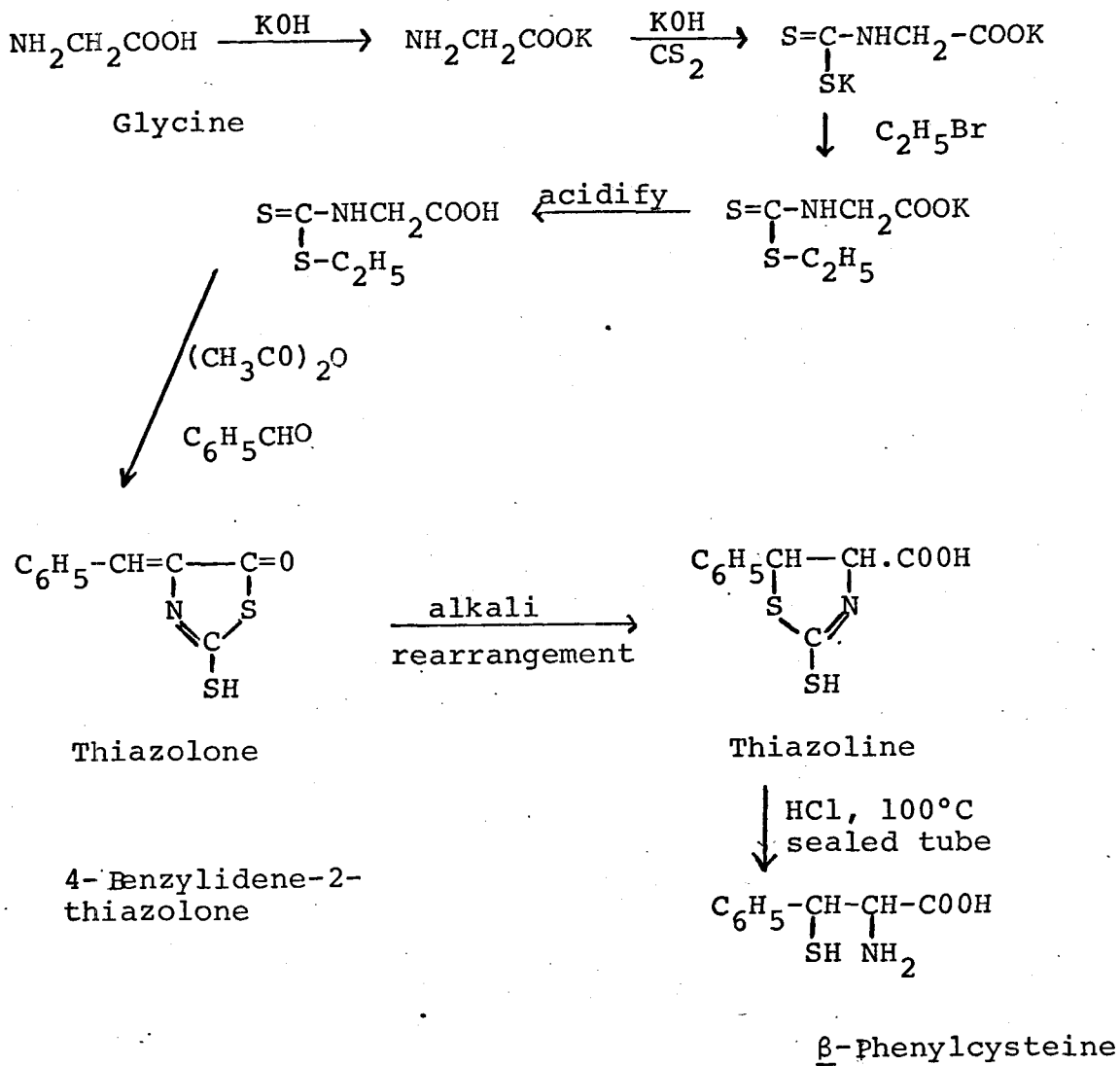
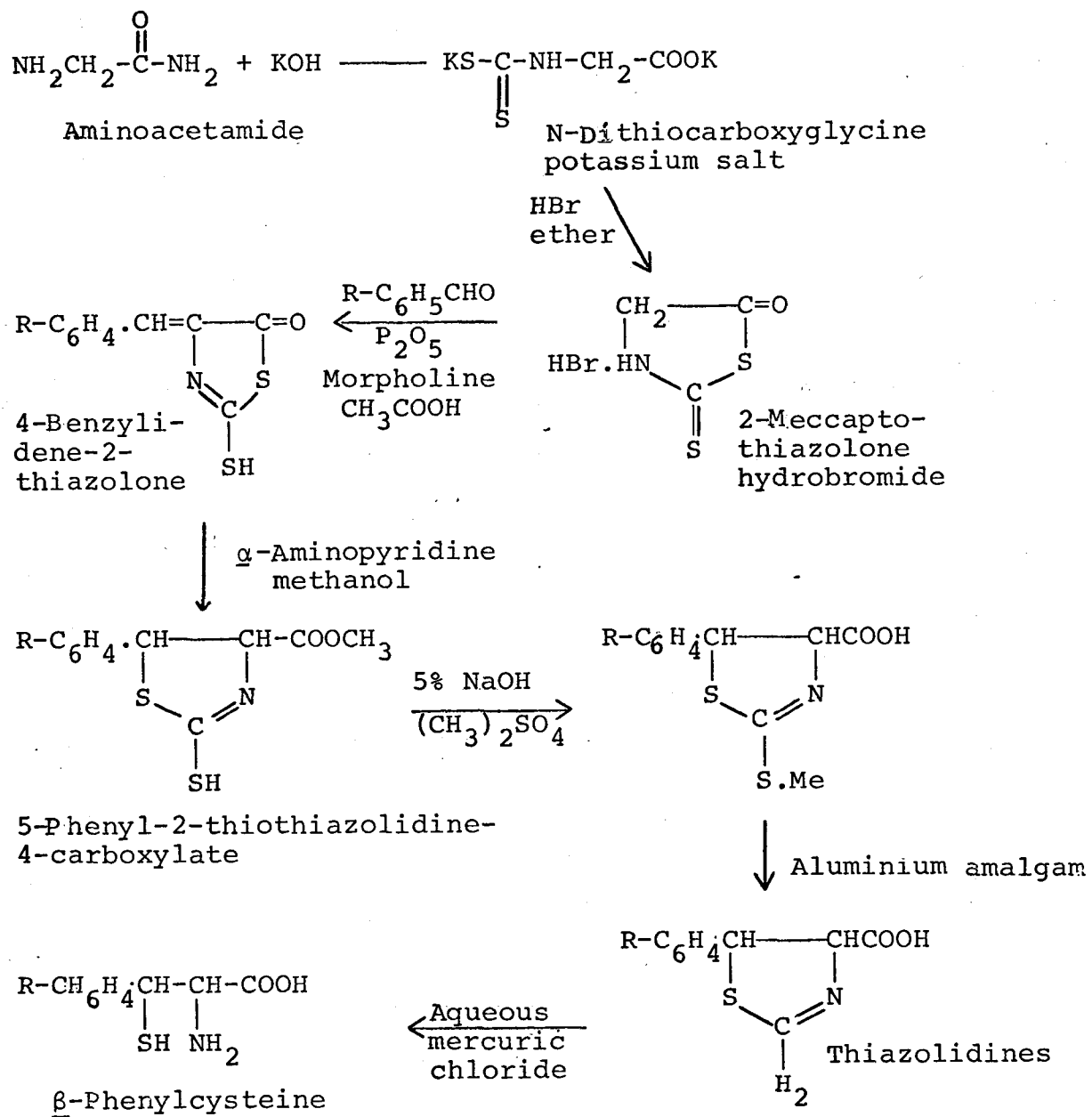


FIGURE 8



condensation reaction between aldehydes or ketones and compounds containing activated methylene groups in the presence of an organic base, to synthesize oxazolones. Treatment of oxazolones with concentrated sulphuric acid and ethanol gave the α,β -unsaturated compounds. In this synthesis, hippuric acid in presence of sodium acetate, acetic anhydride and para-substituted benzaldehydes were used to prepare substituted oxazolones. Para-substituted- α -benzamidocinnamic acid ethyl esters were synthesized from these oxazolones (See Figure 1).

The condensation of dichloroethioacetic acid with these substituted cinnamates was accomplished with 40-47% yield. The constitution and configuration of S-dichloroacetyl-N-benzoyl- β -phenylcysteine ethyl ester has been proved by Sicher and co-workers (23) by alcoholysis and acetylation of the latter compound.

The reduction of the adduct was accomplished by the Meerwein-Ponndorf-Verley reaction (30) which uses aluminium isopropoxide. The yields obtained were satisfactory (70%). The mechanism of these reactions have been discussed in the literature (30).

The synthesis of cystine derivatives was accomplished by oxidation of cysteine derivatives with 0.2N iodine solution in dioxane. Yields varied from 40-85% for the various compounds synthesized. The poor yields were due to large losses during recrystallization steps. No attempt was made to recover the material from the mother-liquor.

The above procedure was found to be more advantageous for the preparation of N-benzoyl-threo- β -p-substituted-phenylcysteine ethyl esters, than the ones from Heilbron and co-workers. (38 - 43).

B. Polarographic Investigation of β -Substituted Phenylcysteine Derivatives

A quantitative method for the determination of derivatives of substituted phenylcysteines and cystines was developed using a modified procedure of Thibert et al. (18,19) which gave molecular weights within $\pm 2\%$. These results were not reported here, since only a duplicate analysis was run on some of them.

A quantitative polarographic method was thought to be feasible due to previous work with alpha-substituted cystines (1). Diffusion current plots vs. concentration showed a linear relationship. This indicated that a rapid and accurate polarographic determination can be carried out without any complication using 0.1M sodium acetate in methanol as the supporting electrolyte at an apparent pH 9.7.

No pH dependency studies were carried out, since a nonaqueous medium was used. Only the apparent pH of the supporting electrolyte was measured. No maxima were observed on the polarograms, therefore, no maximum suppressor was used. The shapes of the polarograms were similar, without a prewave, to the case of cystine and alpha-substituted cystines except that the latter had a

prewave (1, 35).

In order to determine whether the reduction of substituted β -phenylcystine derivatives was reversible, plots of E vs $\log (i_d - i)/i^2$ were made (See Figure 5). All of the amino acid derivatives gave a straight line whose slopes are given in Table VI. Values ranging from 0.0246-0.0185 were obtained (Figure 5). The theoretical slope of 0.0295 indicates a reversible reduction reaction involving two electrons. Experimental values are not very close to the theoretical values and so one can predict, a nonreversible reduction reaction in case of these substituted amino acids except in the case of *N*-benzoyl-threo- β -phenylcystine ethyl ester.

Diffusion coefficients were calculated by substitution of the experimental values into Ilkovic's equation. Table V shows values which lie between $6.75-7.85 \times 10^{-6} \text{ cm}^2 \text{ sec}^{-1} \times 10^6$ for phenyl-, *p*-methoxyphenyl- and *p*-chlorophenylcystine derivatives, while $5.489 \times 10^{-7} \text{ cm}^2 \text{ sec}^{-1} \times 10^6$ was found for the *p*-nitrophenylcystine derivative. The value reported for cystine is $5.70 \times 10^{-6} \text{ cm}^2 \text{ sec}^{-1} \times 10^6$, which is less than that of β -substituted phenylcystine derivatives. Alpha-substituted cystines have diffusion coefficients in the range of $4.87-3.27 \times 10^{-6} \text{ cm}^2 \text{ sec}^{-1} \times 10^6$, which is less than cystine as well as β -substituted phenylcystine derivatives. This could be due to the difference in structure and the use of a non-aqueous medium.

Thus far, the discussion has been mainly concerned with polarographic determinations. Consideration will now be given to the correlation between reaction rate, half-wave potential and the structure of the substituent (the substituent constant - σ). Hammett (8) has defined the substituent constant by the following equation:

$$\log k/k^{\circ} = \sigma\rho$$

where k is the dissociation constant of substituted benzoic acids and k° is the dissociation constant of benzoic acid.

ρ = the reaction constant, which depends on the reaction, the condition, and the nature of the side chain.

It is preferable to compare the rates of the series of reactions with the equilibrium constants of the same series. Since ionization constants are proportional to the equilibrium constants, they could be substituted for equilibrium constants. However, the substituent constants for a particular electron-withdrawing group, applicable to most reactions, does not give good results when used with reactions of other series. Therefore, special substituent constants have been defined by Jaffé (9) and Taft and Lewis (7).

In this study, for the comparison of the effect of substituents on the reduction of cystines, two model compound systems were selected. Equilibrium constants in the two series-ethyl p -substituted phenyl acetate and ethyl p -

substituted cinnamates—were taken from the data of alkaline hydrolysis of these esters (45).

Table X and Figure 9 shows the large changes in reaction rate and equilibrium constants of these esters due to the substituent effect. They correlate quite satisfactorily. No polarographic data were available for this series, which enable us to compare the data of these esters with N-benzoyl- β -p-substituted cystine ethyl esters. p-Phenyl-, p-methoxyphenyl-, p-chlorophenyl- and p-nitrophenylcystine ethyl esters show very little difference (ca 17mV) between their half-wave potentials. However, a strong electron withdrawing group (like NO₂) is required to show this small (17mV) change in half-wave potential. One can, therefore, conclude (Figure 10; Table XI) that there is no apparent correlation between half-wave potential and the structure of the substituent (substituent constants σ^*). There is a possibility that the value of the p-chloro- derivative is lower than one would expect.

In order to show that $E_{1/2}$ values might bear some significant relationship to spectroscopic data (UV), the spectra of these cystine derivatives were measured in methanol. Since no appreciable differences in the wave length of the absorption maxima or shoulders as a function of half-wave potentials were found, no relationship study for these values were carried out (See Table IX).

TABLE X
EQUILIBRIUM CONSTANTS OF p-SUBSTITUTED PHENYL ACETATE
AND p-SUBSTITUTED CINNAMATES*

Acid	k	σ_p	log k
Ethyl phenyl acetate	0.639	0.00	-.1945
Ethyl <u>p</u> -methoxyphenyl acetate	0.580	-0.27	-.2366
Ethyl <u>p</u> -chlorophenyl acetate	1.62	+0.23	+.2095
Ethyl <u>p</u> -nitrophenyl acetate	4.57	+0.78	+.6599
Ethyl <u>p</u> -aminophenyl acetate	0.325	-0.66	-.4881
Ethyl cinnamate	0.106	0.00	-.9747
Ethyl <u>p</u> -methoxycinnamate	0.0444	-0.27	-2.3526
Ethyl <u>p</u> -chlorocinnamate	0.215	+0.23	-.6676
Ethyl <u>p</u> -nitrocinnamate	1.037	+0.78	+.0161
Ethyl <u>p</u> -methylcinnamate	0.0754	-0.17	-2.1226

ρ for phenylacetic acid = 0.489 at 25°C (-log k = 4.297)

and for Ar-CH₂CH₂COOH = 0.212 at 25°C (-log k = 4.551)

* These values were taken from reference (45).

FIGURE 9
PLOT OF LOG k VS. LOG K
FOR ETHYL p-SUBSTITUTED PHENYLACETATE

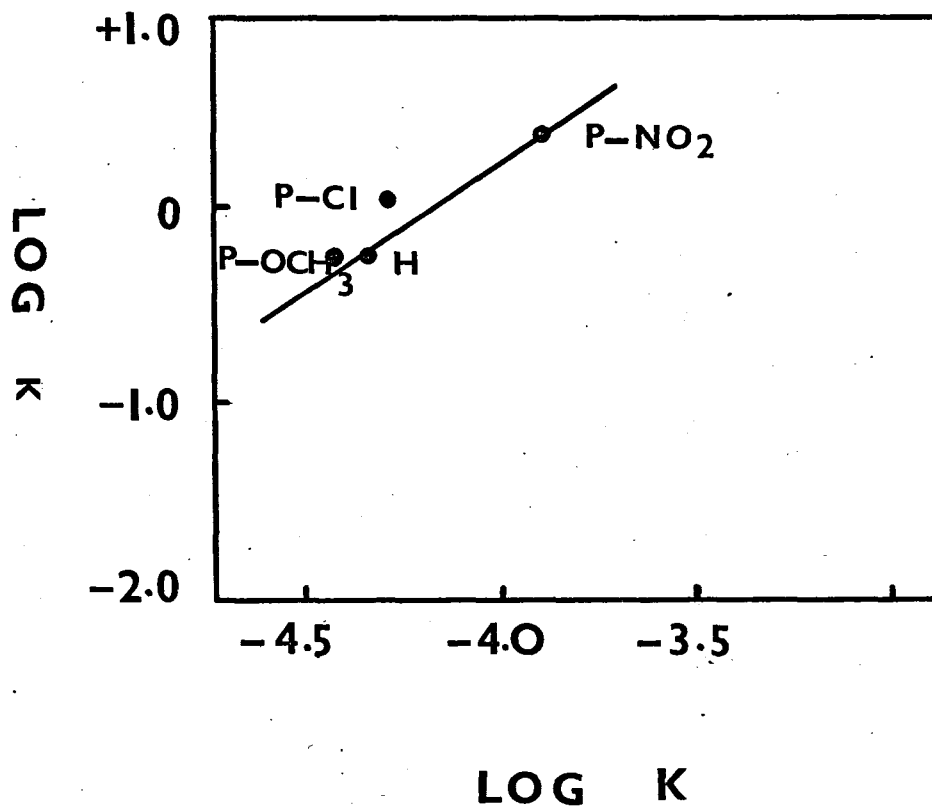


FIGURE 10

PLOT OF $E_{1/2}$ VS. σ^* AND σ_{I} Legend

Data for these plots are taken from half-wave potentials and Taft values (7,8).

Plots are given for phenyl-, p-methoxyphenyl-, p-chlorophenyl- and p-nitrophenylcystine derivatives.

(●) represents σ_{I} and (⊙) represents σ^* values.

FIGURE 10
PLOTS OF $E_{1/2}$ Vs. σ^* AND σ_I

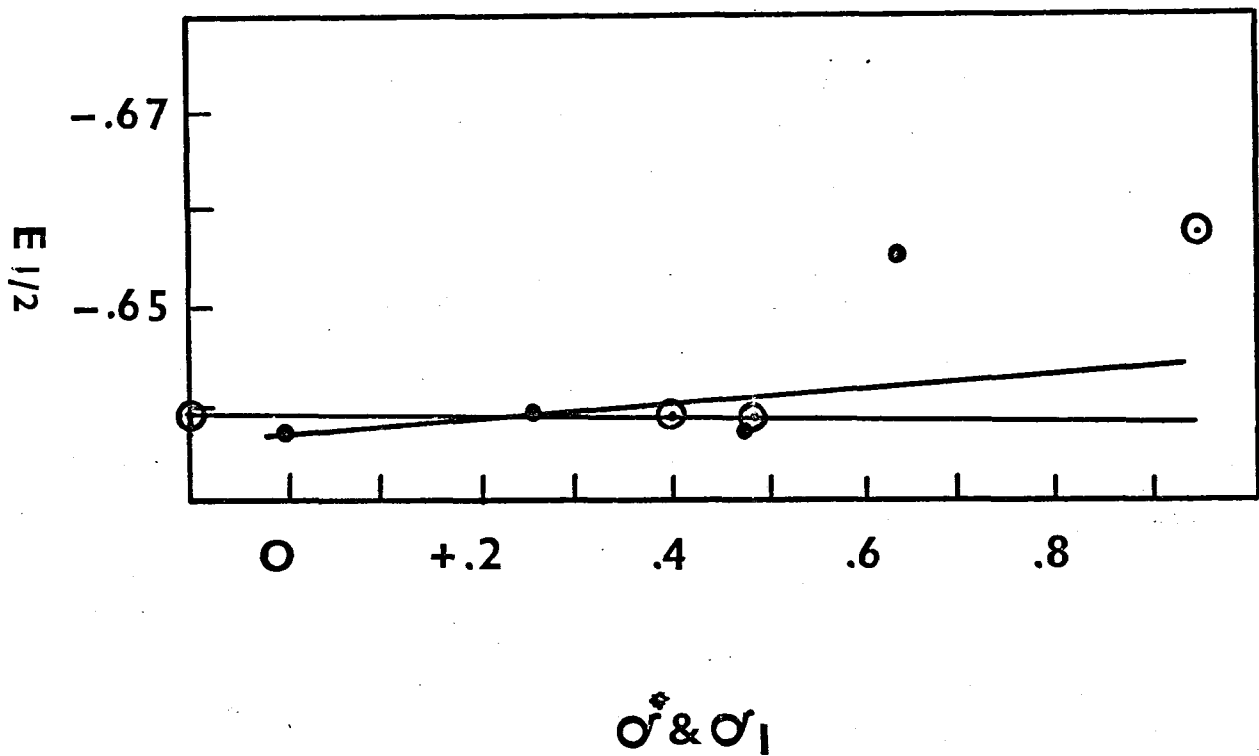


TABLE XI
 HALF-WAVE POTENTIALS AND σ_I ; σ^*

Amino Acid	Half-Wave Potential	σ_I Inductive Const.	σ^*
N-Benzoyl-threo- β - p-phenylcystine ethyl ester	-0.638	0.000	+0.49
N-Benzoyl-threo- β - p-methoxyphenylcystine ethyl ester	-0.639	+0.250	-0.10
N-Benzoyl-threo- β - p-chlorophenyl- cystine ethyl ester	-0.638	+0.470	+0.40
N-Benzoyl-threo- β - p-nitrophenyl- cystine ethyl ester	-9.655	+0.630	+0.95

σ_I Substituent constant (inductive)

σ^* Taft constant (polar substituent).

CHAPTER V

SUMMARY AND CONCLUSIONS

A polarographic method was established for the determination of N-benzoyl- β -phenyl-, N-benzoyl- β -p-methoxyphenyl-, N-benzoyl- β -p-chlorophenyl-, and N-benzoyl- β -p-nitrophenylcystine ethyl esters. All determinations were carried out in 0.1M sodium acetate in methanol at an apparent pH of 9.7. A straight-line correlation was established between the diffusion current and the concentration. The concentrations measured were between 1×10^{-5} — 1×10^{-4} M. Slopes of the logarithmic analysis of $(i_d - i)/i^2$ versus E gave values ranging from 0.0245 - 0.0185. These values show that the reduction is nonreversible. Corrected values of half-wave potentials were determined from plots of voltage versus $\log (i_d - i)/i$.

Absorption characteristics of these compounds ($n \rightarrow \sigma^*$ transition) were measured in methanol and an attempt to establish a correlation between these values and half-wave potentials was attempted. Since there was a very little difference in half-wave potentials for these compounds, no correlation was found between half-wave potentials and the Taft's values (substituent constants).

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