Synthesis and polarographic studies of beta-substituted phenylcystine derivatives

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The synthesis of *N*-benzoyl-*threo*-β-*p*-substituted phenylcystine ethyl esters via azlactone intermediates is reported. A polarographic method for their determination using 0.1 *M* sodium acetate in anhydrous methanol as the supporting electrolyte was investigated. The relationship between the half-wave potential and the Taft polar substituent constant, σ*, was studied.

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Introduction

Polarographic investigations of cystines and alpha-substituted cystines (1, 2) have prompted further experiments with substituted cystines because studies with alpha-substituted cystines revealed that no direct correlation with the Taft polar substituent constants existed. This may have been due in part to the differing steric and polar effects of the various groups in the alpha position on the reduction of the disulfide linkage. It became of interest to study only the polar effects of different groups in the beta position of various para-substituted phenylcystine derivatives on the half-wave potentials, the diffusion current, the diffusion coefficient, and on the general shapes of the polarograms, since the para-substituted phenylcystines would have rather constant steric factors. Studies of this type on the effect of the substituent on the half-wave potential have been reported by Zuman (3).

Experimental

Materials and Methods

The Sargent (E. H. Sargent and Co.) model XVI polarograph with a Sargent model A IR compensator was employed for this study. A special type of micro-H-cell (a modification of the H-cell of Arthur and Lyons (4)) was used. All the measurements were carried out in non-aqueous methanol using a methanolic saturated calomel in the working cell and an aqueous saturated calomel reference electrode. The characteristics of the capillary used were: $m = 1.426$ mg s^{-1}; $t = 5.6$ s; $m^{2/3}t^{1/6} = 1.689$ $mg^{2/3}$ $s^{-1/2}$ at -0.640 V. The height of the mercury column was 53.5 cm. A conductivity bridge (model RC 16 B2, Industrial Instruments, Inc.) was used to measure the cell resistance. A Fluke model 825A differential voltmeter was used to measure the potential against the aqueous saturated calomel electrode. The supporting electrolyte used was 0.1 *M* sodium acetate in anhydrous methanol. No maximum suppressor was required. No damping was used in this study.

p-Substituted benzaldehydes used as starting materials for the synthesis of *N*-benzoyl-*threo*-β-*p*-substituted cystine ethyl esters were obtained from Fisher Scientific Co. The substituted azlactones were prepared according to the procedure of Erlenmeyer *et al.* (5–9) from the corresponding substituted benzaldehydes by condensation with hippuric acid in the presence of anhydrous sodium acetate and acetic anhydride. On treatment of the various azlactones with sulfuric acid and ethanol, the corresponding substituted ethyl-α-benzamidocinnamates were obtained. These cinnamates condensed readily with dichlorothioacetic acid according to the method of Sicher *et al.* (10) to the corresponding *N*-benzoyl-*S*-dichloroacetyl-*threo*-β-*p*-substituted phenylcysteine ethyl esters. The reduction of these compounds via the Meerwein–Ponndorf–Verley (11) reaction gave the corresponding *N*-benzoyl-*threo*-β-*p*-substituted cysteine ethyl esters. The phenyl-, *p*-chlorophenyl-, *p*-methoxyphenyl-, and the *p*-nitrophenylcystine derivatives were prepared from the corresponding substituted cysteine derivatives by oxidation. All melting points reported are uncorrected. Infrared (i.r.) spectra used to identify reaction products were obtained on a Beckman IR 10 spectrophotometer. The microanalyses were performed by Schwarzkopf Microanalytical Laboratory, 56-19 37th Avenue, Woodside, N.Y. 11377, U.S.A.

Synthesis

Compounds which had been previously reported were synthesized and characterized using appropriate literature references. Procedures for preparing new compounds are listed with the physical properties, analyses, and yields. The sequence of reactions used for the various syntheses is outlined in Scheme 1. The physical and analytical data for the various compounds are summarized in Table 1.

Previously Prepared Compounds

2-Phenyl-4-benzylidene azlactone, m.p. 164–166 °C; lit. m.p. 165–166 °C (5–9); yield 75%.

2-Phenyl-4-*p*-methoxybenzylidene azlactone, m.p. 155–156 °C; lit. m.p. 156.5 °C (5–9); yield 42%.

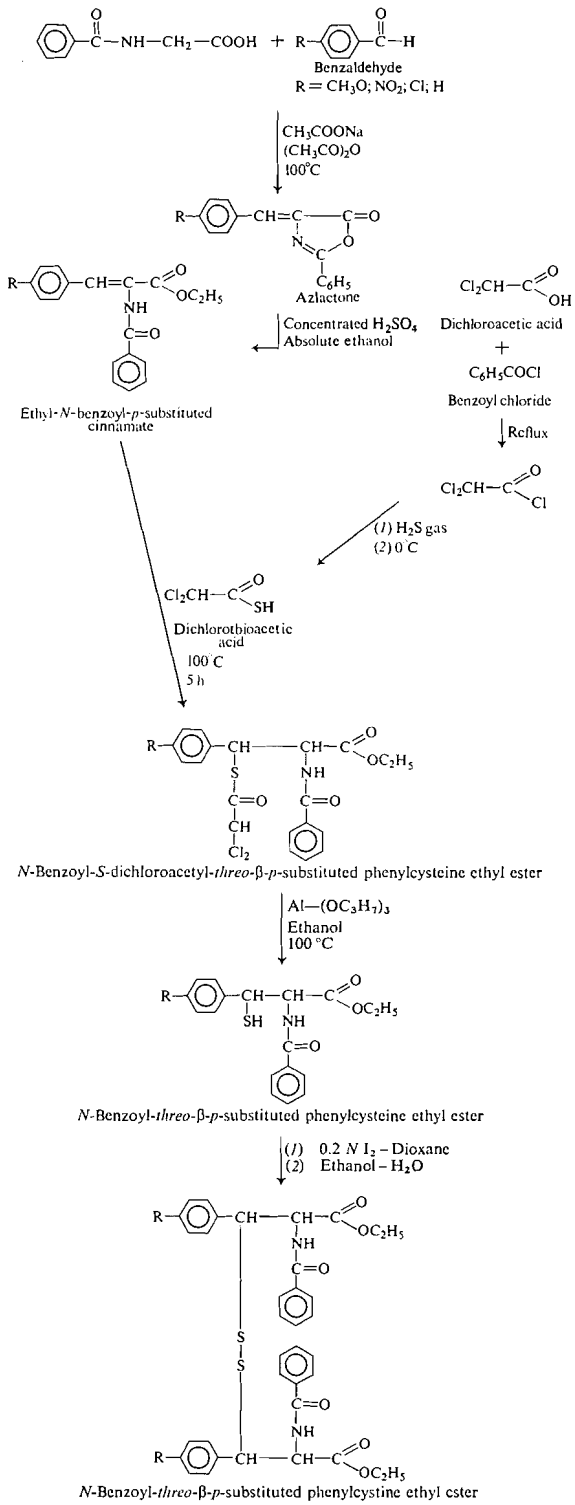
Ethyl-α-benzamidocinnamate, m.p. 148–149 °C; lit. m.p. 149 °C (5–9); yield 85%.

Ethyl-α-benzamido-*p*-nitrocinnamate, m.p. 165–166 °C; lit. m.p. 165–166 °C (10); yield 80%.

N-Benzoyl-*S*-dichloroacetyl-*threo*-β-*p*-phenylcysteine ethyl ester, m.p. 128–131 °C; lit. m.p. 128–131 °C (10); yield 40%.

N-Benzoyl-*S*-dichloroacetyl-*threo*-β-*p*-nitrophenylcysteine ethyl ester, m.p. 142–143 °C; lit. m.p. 142 °C (10); yield 38%.

N-Benzoyl-*threo*-β-phenylcysteine ethyl ester, m.p. 125–126 °C; lit. m.p. 125–126 °C (10); yield 70%.



Synthesis of beta-substituted phenylcystine derivatives

SCHEME 1

TABLE 1

Properties of compounds prepared*

Type of compound	Group R	Yield (%)	Melting point (°C)	Reference	Analysis									
					Found (%)					Calculated (%)				
					C	H	N	S	Cl	C	H	N	S	Cl
2-Phenyl-4-*p*-benzylidene azlactone	H†	75	165–166	5–9										
	OCH_3†	42	155–156	5–9										
	Cl	55	195–196	—	67.80	3.76	4.92		12.35	67.60	3.87	4.93		12.32
	NO_2	67	235–237 (dec.)	—										
Ethyl-α-benzamido-cinnamates	H†	85	148–149	5–9										
	OCH_3	91.6	146–147	—	70.07	5.80	4.28			70.15	5.84	4.30		
	Cl	73.3	149–150	—	65.45	4.97	4.01		10.56	65.65	4.86	4.25		10.63
	NO_2†	80	165–166	10										
Adduct with dichloro-thioacetic acid	H†	40	128–131	10										
	OCH_3	31	108–109	—										
	Cl	30.9	133–134	—										
	NO_2†	38	142–143	10										
β-Phenylcysteine ethyl esters	H†	70	125–126	10										
	OCH_3	41.6	139–140	—	62.42	6.04	4.06	9.40		62.49	6.02	4.02	9.17	—
	Cl	37	147–148	—	59.16	5.25	4.04	8.87	9.39	59.40	4.95	3.85	8.81	9.78
	NO_2	75	134–135	—	49.22	4.69				48.88	5.18			
β-*p*-Substituted phenyl-cystine ethyl esters	H†	75	135–138	—										
	OCH_3	70	153–155	—										
	Cl	60	198–200	—										
	NO_2	40	230–232	—										

*The analyses reported in this table refer only to the newly synthesized compounds.
†These compounds have been reported in the literature.

Intermediates

Azlactone Syntheses

Azlactones (2-phenyl-4-*p*-nitrobenzylidene azlactone and 2-phenyl-4-*p*-chlorobenzylidene azlactone) were prepared by the method of Erlenmeyer *et al.* (5–9), which involves the reaction of the appropriate substituted benzaldehydes (0.33 mole) with hippuric acid (0.33 mole) in the presence of anhydrous sodium acetate (0.33 mole) and acetic anhydride (0.83 mole). The mixture was refluxed for 1.5 h, and the resulting crude product was filtered, washed with alcohol, and recrystallized from benzene. The yields obtained were 67 and 55%, respectively. The corresponding m.p. were 235–237 °C (dec.) and 195–196 °C.

Cinnamate Syntheses

The substituted cinnamates (ethyl-α-benzamido-*p*-methoxycinnamate and ethyl-α-benzamido-*p*-chlorocinnamate) were synthesized according to the procedure of Erlenmeyer *et al.* (5–9). A suspension of the appropriate azlactones (0.05 mole) was refluxed with a mixture of absolute ethanol (2.1 moles) and concentrated sulfuric acid (5 ml) for 3–4 h. After removal of the excess ethanol, the white residue was washed with water until free of any traces of sulfuric acid. The product was recrystallized from ethanol. The yield and m.p. were 91.6%, m.p. 146–147 °C for the *p*-methoxy derivative and 73.3%, m.p. 149–150 °C for the *p*-chloro derivative.

Adducts of Dichlorothioacetic Acid with the Ethyl Cinnamates (10)

A mixture of ethyl-α-benzamido-*p*-substituted cinnamate (0.023 mole) and dichlorothioacetic acid (10 ml) was refluxed on a steam bath for 5 h. On addition of ether, scratching immediately induced separation of white crystals. The crystals were washed and recrystallized from ethanol. The yields and melting points of *N*-benzoyl-*S*-dichloroacetyl-*threo*-β-*p*-chlorophenylcysteine ethyl ester and *N*-benzoyl-*S*-dichloroacetyl-*threo*-β-*p*-methoxyphenylcysteine ethyl ester are given in Table 1.

Syntheses of N-Benzoyl-threo-β-p-substituted Phenylcystine Ethyl Esters

The adduct with dichlorothioacetic acid (0.005 mole) was reduced with aluminum isopropoxide (0.005 mole) in ethanol to the corresponding sulfhydryl amino acid derivative, by refluxing for 4 h (11). On evaporation of the excess ethanol and treatment of the residue with 15% tartaric acid (100 ml), a white precipitate was obtained. This was extracted with ether, the ether layer was dried, and on evaporation of the ether a white solid was obtained. This was recrystallized from isopropanol. The yields and melting points for *N*-benzoyl-*threo*-β-*p*-methoxyphenylcysteine ethyl ester, *N*-benzoyl-*threo*-β-*p*-chlorophenylcysteine ethyl ester, and *N*-benzoyl-*threo*-β-*p*-nitrophenyl derivative are reported in Table 1.

Syntheses of N-Benzoyl-β-p-substituted Phenylcystine Ethyl Esters

The appropriate *N*-benzoyl-*threo*-β-*p*-substituted phenylcysteine ethyl ester (250 mg) was dissolved in 40 ml of dioxane and titrated with 0.2 *N* iodine solution. The solution was evaporated to dryness and the resulting residue treated with 5–10 ml of ethanol–water mixture. Filtration and recrystallization yielded the pure cystine derivative in 40–75% yield. The yields and melting points for *N*-benzoyl-*threo*-β-phenylcystine ethyl ester, *N*-benzoyl-*threo*-β-*p*-methoxyphenylcystine ester, *N*-benzoyl-*threo*-β-*p*-chlorophenylcystine ethyl ester, and *N*-benzoyl-*threo*-β-*p*-nitrophenylcystine ethyl ester are listed in Table 1.

Polarographic Procedure

Stock solutions of beta-substituted phenylcystine derivatives were prepared by dilution of solutions made from accurately weighed portions of the appropriate substituted phenylcystines. For polarography, the solutions were prepared using aliquots of the stock solution and diluting to the proper volume with stock electrolyte solution (0.2 *M* sodium acetate in anhydrous methanol). The solutions were transferred to the polarographic cells and nitrogen (purified by passage through a gas-washing bottle containing copper turnings and a 1:1 ammonium hydroxide solution saturated with ammonium chloride and another one containing concentrated sulfuric acid) was bubbled through for 15–20 min. All polarograms were run at 25 ± 0.1 °C. The apparent p*H* of the solutions used for polarography was 9.7.

Results

Effects of Concentration on the Diffusion Current

A linear relation of the diffusion current to the concentration was obtained (Table 2). No prewave was observed as reported by Kalousek *et al.* (12) for the case of the polarographic reduction of cystines and α-substituted cystines as shown by Thibert and Walton in an aqueous medium (2). All of the amino acids gave only one wave except the *p*-nitro derivative, where two waves were observed. The first wave in this case represents the disulfide reduction (this was confirmed by observing the reduction of *p*-nitrobenzaldehyde and ethyl-α-benzamido-*p*-nitrocinnamate). The true half-wave potentials were calculated according to the method of Taylor and Smith (13).

Diffusion Coefficient and Reversibility of the Reaction

By substitution in the Ilkovic equation, the

TABLE 2

Effect of concentration on diffusion current*

Amino acid† concentration (mole/l)	Diffusion current (μA)			
	H	Methoxy	Chloro	Nitro‡
1×10^{-4}	0.541	0.587	0.491	1.192
8×10^{-5}	0.448	0.487	0.402	1.078
5×10^{-5}	0.310	0.304	0.281	0.848
3×10^{-5}	—	—	—	0.626
2×10^{-5}	—	0.141	0.117	—
1×10^{-5}	0.070	—	—	—

*All the values are an average of three determinations and are uncorrected for residual current.

†Amino acid–appropriate *N*-benzoyl-*threo*-β-*p*-substituted phenylcystine ethyl ester.

‡Diffusion current measured from the first wave.

TABLE 3
Properties of some amino acids

Amino acid*	Diffusion coefficient ($cm^2\ s^{-1} \times 10^6$)	Half-wave potential (V)	Slope of plots of E vs. $\log (i_d - i)/i^2$
β-Phenyl-	7.24	−0.638	0.0246
β-*p*-Methoxy-phenyl-	6.33	−0.639	0.0208
β-*p*-Chloro-phenyl-	6.24	−0.638	0.0185
β-*p*-Nitro-phenyl-	50.8	−0.655	0.0212

*Amino acid derivatives–appropriate *N*-benzoyl-*threo*-β-*p*-substituted cystine ethyl ester. The amino acid concentration employed in the polarographic measurements was 5×10^{-5} *M*.

diffusion coefficients for the beta-substituted phenylcystine derivatives were calculated. They were found to be between 6.24 to 50.8 $\times$ 10^{-6} $cm^2\ s^{-1}$. The reversibility of the reaction was tested by plotting E vs. $\log (i_d - i)/i^2$ (1). The slopes of these plots are given in Table 3 (all current values used have been corrected for residual current).

Half-wave Potential

Determination of half-wave potentials was carried out in 0.1 *M* sodium acetate in anhydrous methanol for the phenyl-, *p*-methoxyphenyl-, *p*-chlorophenyl-, and *p*-nitrophenylcystine derivatives. True half-wave potentials were measured from a plot of E vs. $\log (i_d - i)/i$ for a particular polarogram where all current values were corrected for residual current (13).

Discussion

A polarographic method has been developed for the quantitative determination of derivatives of substituted phenylcystines. A quantitative polarographic method was thought to be feasible due to previous work with alpha-substituted cystines (2). 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 0.1 *M* sodium acetate in methanol as the supporting electrolyte at an apparent p*H* 9.7.

No p*H* dependency studies were carried out, since a non-aqueous medium was used. Only the apparent p*H* 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 to those of cystine and alpha-substituted cystines but different from them in having no prewave (1, 2).

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. All of the amino acid derivatives gave a straight line whose slopes are given in Table 3. Values ranging from 0.0246–0.0185 were obtained. 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 it cannot be determined whether a reversible or a nonreversible reduction reaction occurs in the case of these substituted amino acids.

Although straight lines were obtained for plots of $\log (i_d - i)/i^2$ vs. E, the data obtained at constant p*H* and constant concentration of the amino acids in no way suggest any variation of $E_{1/2}$ with concentration of the amino acids as would have been predicted by this type of relationship (i.e., $E_{1/2}$ shows no change with concentration as observed from the numerous polarograms run). This type of observation is probably explained by the fact that the mechanism is more complex than anticipated, as based on a comparison with an apparent two-electron reduction step in the case of cystine. Furthermore, the case of the *p*-nitrophenylcystine derivative is particularly difficult to interpret with regards to the abnormally high diffusion current obtained for the reduction of the disulfide. It appears that the nitro group in some way enhances the reduction of the disulfide thereby giving the latter an abnormally high diffusion current. At this stage, therefore, it is not possible to speculate on the complex nature of the reduction for these types of disulfides. The values of $E_{1/2}$ are valid under the experimental conditions reported here.

Diffusion coefficients were calculated by substitution of the experimental values into Ilkovic's equation. Table 3 shows values which lie between 7.24 to 6.24 $\times$ 10^{-6} $cm^2\ s^{-1}$ for phenyl-, *p*-methoxyphenyl-, and *p*-chlorophenylcystine derivatives, while 50.8 $\times$ 10^{-6} $cm^2\ s^{-1}$ was found for the *p*-nitrophenylcystine derivative. The value reported for cystine is 5.70 $\times$ 10^{-6} $cm^2\ s^{-1}$, which is less than that of β-substituted phenylcystine derivatives. Alpha-substituted cystines have diffusion coefficients in the range of 4.87 to

3.27×10^{-6} cm^2 s^{-1}, 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 the half-wave potential and the structure of the substituent (the substituent constant σ*). Hammett (14) 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. Rho equals the reaction constant, which depends on the reaction, the condition, and the nature of the side chain. 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 Jaffe (15), Taft and Lewis (16), and several others have been summarized by Swain and Lupton (17).

An attempt was made to correlate the half-wave potentials of the various β-phenylcystine derivatives with several of the substituent constants summarized by Swain and Lupton (17) and the Taft and Lewis constants (16). *p*-Phenyl-, *p*-methoxyphenyl-, *p*-chlorophenyl-, and *p*-nitrophenylcystine ethyl esters show very little difference (ca. 17 mV) between their half-wave potentials. Only a strong electron-withdrawing group (like NO_2) can effect this small (17 mV) change in half-wave potential. One can, therefore, conclude that there is no apparent correlation between half-wave potential and the structure of the substituent (substituent constant e.g. σ*). There is a possibility that the value of the *p*-chloro-derivative is lower than one would expect (possibly due to resonance effects).

In the final analysis, it appears that substitution in disulfides must occur directly on the sulfur for any correlation between $E_{1/2}$ and polar substituent constants (3). Attempts at correlation of polar constants such as σ* in the alpha-substituted cystines was not successful perhaps due to both steric and polar effects since substitution of the alpha carbon did not maintain a constant steric environment (2).

In the beta-substituted cystines, however, the steric factors were kept relatively constant throughout the series. Since no direct correlation of $E_{1/2}$ with any of the various polar substituent constants was obtained, it can be concluded that direct attachment to the sulfur of the disulfide linkage is required for manifestations of polar effects (3). Even one carbon interposed between the electroactive group is enough to obviate the polar effects. In fact, so good is the insulation of the electroactive group from the para-substituent on the phenyl group of the beta-substituted phenylcystines, that only a nitro group with its very large polar effect can cause any change in the half-wave potential.

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