STABILITY OF COMPLEXES OF α-CYCLODEXTRIN AND SYMMETRICAL 4,4' - DISUBSTITUTED BIPHENYL COMPOUNDS

by

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STABILITY OF COMPLEXES OF α-CYCLODEXTRIN AND SYMMETRICAL 4,4' - DISUBSTITUTED BIPHENYL COMPOUNDS

Andrea Paulson

(Under the supervision of Professor Kenneth A. Connors)

Previous work in this laboratory examined the complexation of α-cyclodextrin (ligand, L) and a series of symmetrical 1,4 - disubstituted benzenes (substrate, S). The constants for SL and SL_2 binding were determined in 0.1 M NaCl aqueous solution at 25° C. A binding model had been propsed to interpet the experimental data. In this symmetrical two-site substrate, the observed stability constants K_{11} and K_{12} are related to the microscopic binding constants by $K_{11} = 2\ K_{x'x}$ and $K_{12} = a_{XX}\ K_{11} / 4$, where $a_{XX} = K_{x'x'} / K_{x'x}$ by definition. Thus, a_{XX} describes the interaction between binding sites.

Other workers have described the factors that affect the interaction parameter. One of those factors is the electronic effect, which results from resonance between the binding sites. Owing to the electronic effect, one would expect to see a correlation between a_{XX} and σ, the Hammett substituent constant. Such a correlation was seen in the benzene series. Another factor, which can oppose the electronic effect, is the repositioning effect, which is dependent on the distance between the two binding sites.

The the work presented in this thesis, K_{11}, K_{12}, and a_{XX} were determined for complexes of α-cyclodextrin and a series of symmetrical 4,4' - disubstituted biphenyl compounds under the same experimental conditions. These compounds were chosen because they are capable of resonance beween sites and because they provide an increased distance between sites, compared to the benzene series.

A good correlation between a_{XX} and σ was seen in the biphenyl series. The slope of the Hammett plot, ρ, was determined and was compared to the ρ value for the benzene series. We conclude that the extent of electon delocalization between binding sites in the biphenyl series is similiar to that in the benzene series in these cyclodextrin complexes.

Approved: _____

K. A. Connors

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date

I INTRODUCTION

Cyclodextrins (cycloamyloses) are oligosaccharides consisting of six or more D (+) - glucopyranose units connected by α-1,4 linkages to form a torus. The cyclodextrins consisting of six, seven, and eight units are termed α–, β–, and γ– cyclodextrin, respectively. Higher homologs exist, but they have not been studied.

The first description of cyclodextrins in the literature was by Villiers in 1891 (1). Many reviews on subsequent work are available (2). Owing to their size and shape, cyclodextrins form inclusion complexes with a variety of suitable substances. This complexing ability has lead to applications in pharmaceuticals (3), chromatography (4), and model enzyme catalysis (5).

A. Physical Properties and Structure

Table I lists some important physical properties of α–, β– and γ– cyclodextrin. Unlike their monomer, D (+) - glucopyranose, cyclodextrins are non-reducing carbohydrates. They are stable in alkaline solution, but are susceptible to acid hydrolysis (17).

The molecular structure of α-cyclodextrin is shown in figure 1. The numbering scheme for the carbon atoms of the glucose units is shown in figure 2.

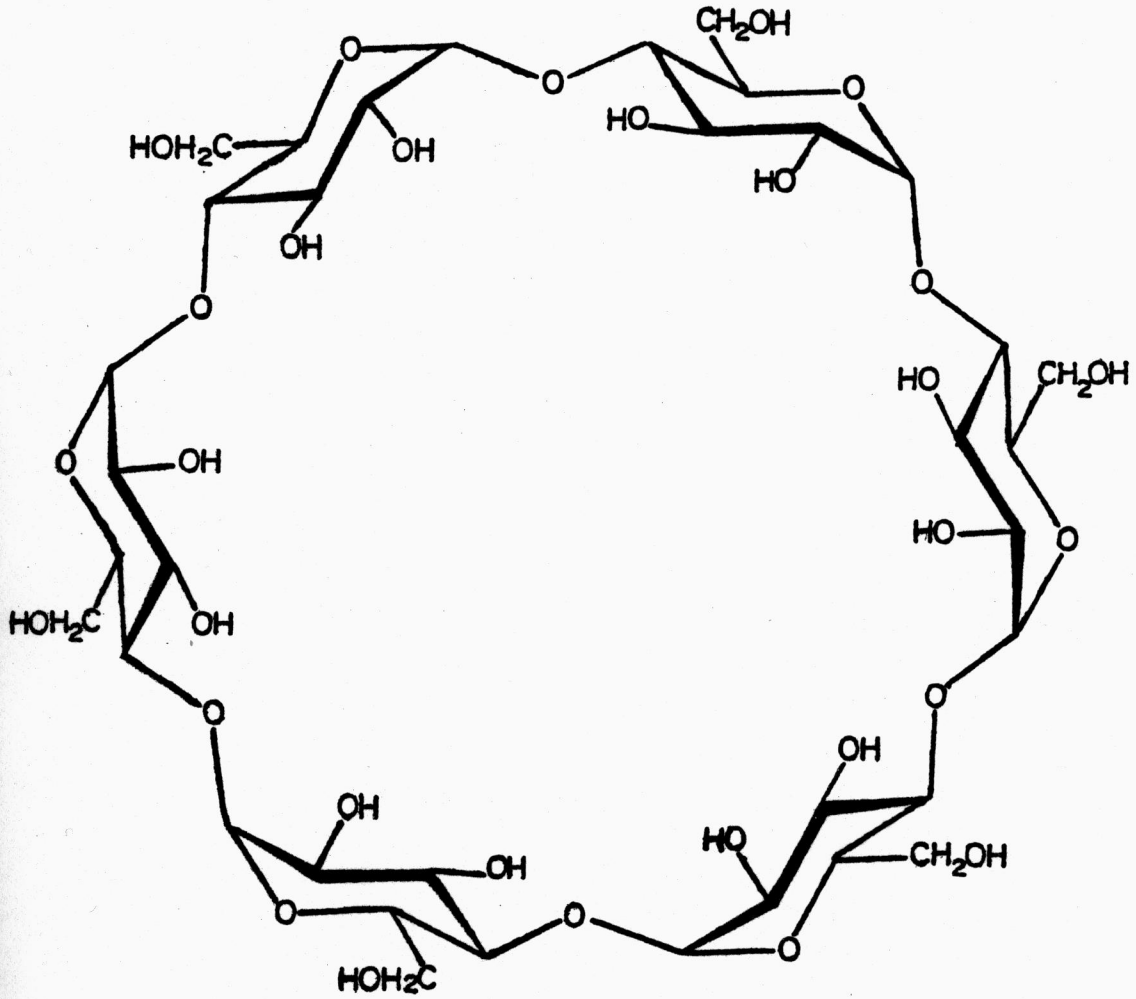
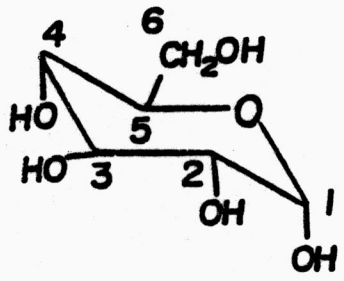


Figure 1. Structure of α-cyclodextrin.

Figure 2

1. Glucose Carbon Numbering Scheme



2. α-(1,4) Linkage

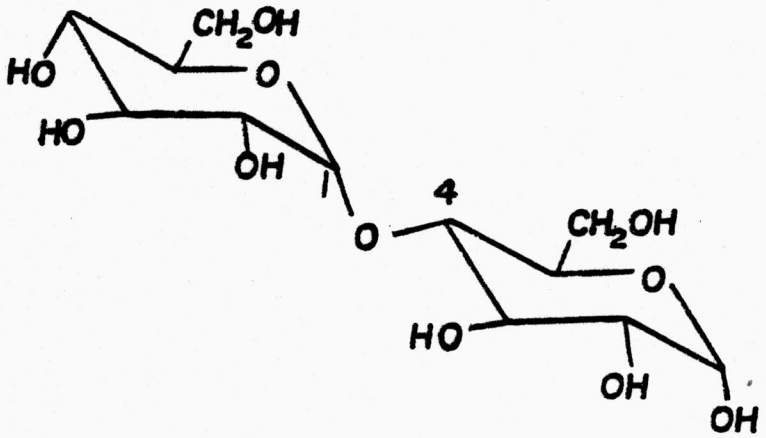


Table I

Physical Properties of Cyclodextrins

	Number of glucose units	Molecular weight	Aqueous solubility @ 25°C (g/100 ml)	Cavity dimensions (Å) internal diameter	depth
α	6	972	11.78[a]	4.7[b] 6.0[c] 4.7-5.2[d]	6.7[b]
β	7	1135	1.850[a]	8[c] 6.0-6.4[d]	7.0[e]
γ	8	1297	21.76[a]	10.0[c] 7.5-8.3[d]	7.0[e]

a ref. 6

b ref. 7, from x-ray analysis

c ref. 8

d ref. 9

e ref. 2b, estimated from Courtald molecular models

Proton nuclear magnetic resonance data of solutions of α–cyclodextrin in dimethyl sulfoxide or D_2O (10, 11, 12), and x-ray crystallographic data of solid α-cyclodextrin (13) are consistent with the following structural features. Each α-D-glucopyranose unit in α-cyclodextrin is in the C1 (chair) conformation. As a result, all six primary hydroxyl groups are located around one rim of the torus, while all twelve secondary hydroxyl groups are located around the other rim. The primary hydroxyl groups are free to rotate about the 5, 6 C-C bond and thus can partially block that entrance to the cavity. The secondary hydroxyl groups do not have this rotational freedom.

Hydrogen bonding exists between the C-2 (secondary) hydroxyl group on one glucose unit and the C-3 (secondary) hydroxyl group of the adjacent glucose unit. This may contribute to the relative rigidity of the torus structure. The interior of the molecule is lined with a ring of glycosidic oxygen linkages between two rings of C-H groups. Therefore, the molecule's interior is non-polar relative to water.

Additional x-ray crystallographic data (14, 15) and crystallographic plus neutron diffraction data (16) provide the following information about the solid α-cyclodextrin-H_2O complex, which is a hexahydrate. Two of the six water molecules reside inside the cavity. These water molecules are hydrogen bonded to primary hydroxyl groups of glucose units 1 and 5, and are hydrogen bonded to each other. This causes glucose units 1 and 5 to be at a different angle to the axis of symmetry, as compared with the other glucose units. This causes the macrocycle to be distorted . In aqueous solution, the ring distortion is retained.

B.Cyclodextrin Complexes

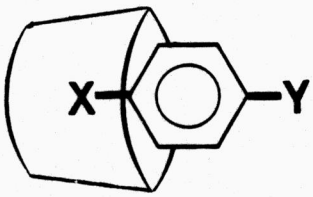
1. Binding forces

Cyclodextrins have been shown to form complexes with a wide variety of molecules. The forces of interaction which stabilize these complexes is not fully understood. The following forces have been proposed: hydrogen bonding (8), hydrophobic interactions (18, 19), release of high energy water (20, 21), relief of ring strain (14, 15),and van der Waals forces (22). Evidence from empirical correlations indicates that , in general, the major binding forces are van der Waals forces (23, 24) and hydrophobic interactions (25, 26). Tabushi (23) proposed a theoretical model in which complex stability was dependent upon several of these forces. An empirical approach to the problem of binding forces was described by Connors and Pendergast (28). This model will be described in detail in a later section.

2. Stoichiometry and Isomerism

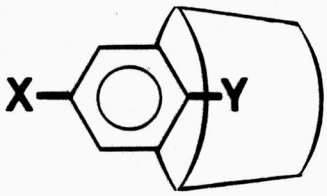
In this work, cyclodextrin will be referred to as the ligand (L), and the molecule complexed by cyclodextrin as the substrate (S). Complex stoichiometry will be described by the ratio of substrate molecules to ligand molecules in a complex.

Because primary hydroxyl groups in α–cyclodextrin can freely rotate and block that entrance to the cavity, 2:1 (S_2L) isomers are not generally seen with this ligand. The occurrence of a 2:1 isomer in the complexation of benzene has been reported recently (46). However , for a typical two-site substrate, we will consider only three possible isomers. Using a 1,4- disubstituted benzene compound as an example, the possible isomers for a two-site substrate and α-cyclodextrin are two SL isomers, **1** and **2**, and one SL_2 isomer, **3**.



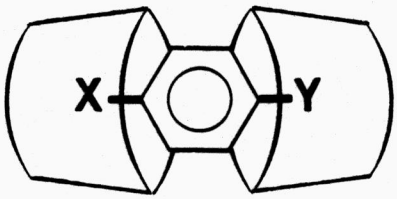
1:1

1



1:1

2



1:2

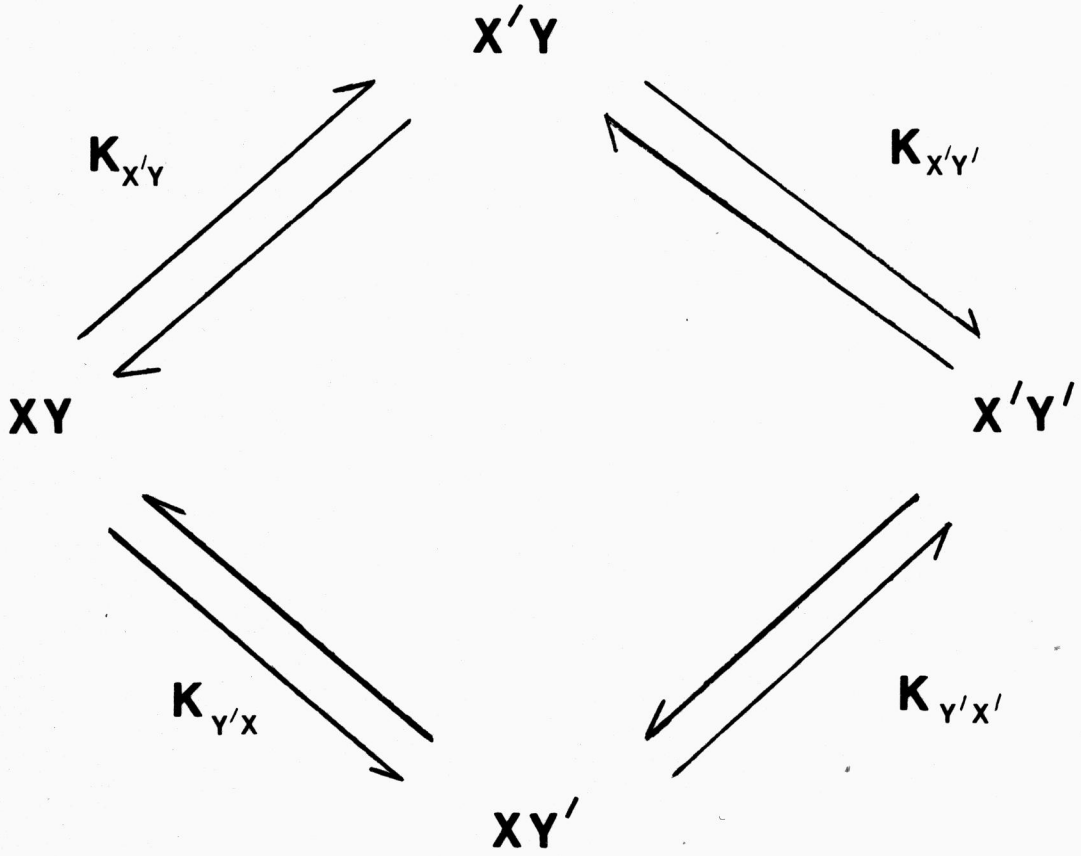
3

II STATEMENT OF THE PROBLEM

A. Binding Model

Previous work in this lab has examined the complexation of α-cyclodextrin and a series of disubstituted benzene compounds (24,28). To aid in the interpretation of experimentally determined equilibrium constants, a binding model has been proposed for substrates with two binding sites (29). That model is represented by Scheme I.

Scheme **I**



In this scheme, the superscript prime indicates a site bound by ligand. The leading substituent, X or Y, was the site that was first bound. $K_{Y'X}$ is the microscopic binding constant for 1:1 binding at the Y site. $K_{Y'X'}$ is the microscopic binding constant for 1:2 binding (at the X site) when the Y site has been previously complexed.

The experimentally determined constants, K_{11} and K_{12}, are related to the microscopic constants by eqs. 1 and 2.

$$K_{11} = K_{X'Y} + K_{Y'X} \qquad (1)$$

$$K_{12} = \frac{K_{X'Y'}K_{Y'X'}}{K_{X'Y'} + K_{Y'X'}} \qquad (2)$$

The model was further developed with the definition of the interaction parameter, a_{XY} (24), as seen in eq. 3.

$$a_{XY} = \frac{K_{Y'X'}}{K_{X'Y}} \qquad (3)$$

One can see that a_{XY} is the ratio of the microscopic constant for binding of the X site when the Y site has been previously complexed to the microscopic constant for binding at the X site when the Y site is unbound. From the ratios of the species concentrations that define the microscopic constsants, one can show that

$$\frac{K_{Y'X'}}{K_{X'Y}} = \frac{K_{X'Y'}}{K_{Y'X}} \qquad (4)$$

Combining eqs. 1,2,3, and 4 gives eq. 5.

$$K_{12} = \frac{a_{XY} K_{X'Y} K_{Y'X}}{K_{X'Y} + K_{Y'X}} = \frac{a_{XY} K_{X'Y} K_{Y'X}}{K_{11}} \qquad (5)$$

An important special case of the binding model occurs when X = Y. The binding constants then become

$$K_{11} = 2 K_{X'X} \qquad (6)$$

$$K_{12} = \frac{a_{xx} K_{11}}{4} \qquad (7)$$

$$a_{XX} = \frac{K_{X'X'}}{K_{X'X}} \qquad (8)$$

a_{XX} can then be estimated from eq. 7.

Previous work in this laboratory by Connors and Pendergast (28) was performed on symmetrical disubstituted benzene compounds. The special case of the binding model in which X = Y was applied to that data. It will also be applied to the data reported in this work.

B. Complex Stability

Connnors, Lin and Wong (24) proposed the following postulates to interpret the complexation of α-cyclodextrin in solution. An increase in complex stability is seen with an increase in site electron density and an increase in site polarizability. A decrease in complex stability is seen with an increase in site polarity.

Connors and Pendergast (28) developed these postulates into a general model that partitions contributions to complex stability. In eq. 9,

$$\Delta G^{\circ}_{X'X} = \Delta G^{\circ}_{MM} + \Delta G^{\circ}_{MS} + \Delta G^{\circ}_{SS} \qquad (9)$$

$\Delta G^{\circ}_{X'X}$ is defined by $-RT \ln K_{X'X}$, where $K_{X'X}$ is the microscopic constant for 1:1 binding to substrate XX. The free energy terms in eq. 9 refer to free energy changes due to medium-medium interactions (ΔG°_{MM}), medium-solute interactions (ΔG°_{MS}), and solute-solute interactions (ΔG°_{SS}).

Medium-medium interactions include the hydrophobic effect. As two solute species associate, they reduce the area of solute-water interface. A reduction in the surface area of water will decrease total free energy. For solvents other than water, a decrease in energy will be seen if the solvent is more polar than the cyclodextrin interior.

Medium -solute interactions include solvation of the substrate and the cyclodextrin. In general, solvation of the substrate is decreased while it is complexed. For a highly polar molecule, a decrease in solvation will increase the total energy of the system. For a non-polar substrate, a decrease in solvation will decrease the energy of the system.

Solute-solute interactions include electrostatic, induction and dispersion forces, and hydrogen bonding. Because α-cyclodextrin has no permanent charge, van der Waals forces between ligand and substrate are attractive forces, and therefore stabilizing. Site polarizability and site electron density will affect the magnitude of these forces.

C. Effects on the Magnitude of a_{XX}

Connors and Pendergast (28) described the influences on the magnitude of the interaction parameter a_{XX}. We see from eq. 8 that if the binding sites are independent (i.e. $K_{X'X} = K_{X'X'}$), then $a_{XX} = 1$. Because a_{XX} is a function of both $K_{X'X}$ and $K_{X'X'}$, the influences on a_{XX} differ from the effects described by eq. 9, which is a function of $K_{X'X}$ only.

We can rewrite eq. 8 as

$$\Delta G^{\circ}_{X'X'} = \Delta G^{\circ}_{X'X} - RT \ln a_{XX} \qquad (10)$$

where $\Delta G^{\circ}_{X'X'} = - RT \ln K_{X'X'}$ and $\Delta G^{\circ}_{X'X}$ is defined as in eq. 9. We can partition the last term in eq. 10 into the following:

$$- RT \ln a_{XX} = \Delta G^{\circ}_{R} + \Delta G^{\circ}_{L\text{-}L} + \Delta G^{\circ}_{E} \qquad (11)$$

The free energy terms refer to changes in free energy due to repositioning (ΔG°_{R}), ligand-ligand interactions ($\Delta G^{\circ}_{L\text{-}L}$), and electronic effects (ΔG°_{E})

1. The repositioning effect

The relative positions of the substrate and ligand in a 1:1 complex are those which produce the minimum total free energy. The position of the three molecules in a

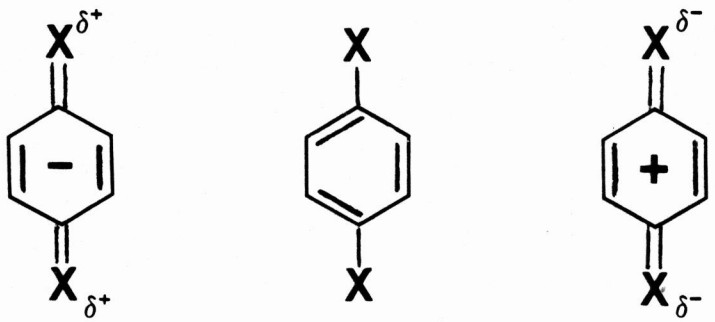
1:2 complex will also minimize total free energy . If a change in relative positions of the molecules in the 1:1 complex is required to optimize the 1:2 complex, the 1:2 complex will be less stable relative to the 1:1 complex. a_{XX} will be decreased as a consequence.

2. Ligand-ligand interactions

In a 1:2 complex, the two facing rims of α-cyclodextrin may attract each other, causing stabilization of the 1:2 complex, relative to the 1:1 complex. This will increase a_{XX}. If the rims should repel each other, this interaction will be accounted for in the repositioning efffect.

3. Electronic effect

A symmetrical disubstituted compound can be considered a hybrid of resonance forms **1** through **3**.



1 **2** **3**

If substituent X is electron donating, resonance form **1**, in which the X site is electron deficient, represents the most probable charge distribution. In 1:1 binding (X' X), the ligand can act as a source of electrons. Partial charge transfer occurs from the ligand to the X' site in X'X, and then to the X site due to resonance. Therefore, the X site in X'X is more electron rich than the X site in XX. Increased site electron density was postulated to be complex stabilizing. Therefore, if X is electron donating, a_{XX} will be increased. If X is electron withdrawing, the ligand can act as a sink for electrons. the opposite drift of charge occurs, and a_{XX} will be decreased.

From eq. 11, we see that if $a_{XX} = 1$, the sites are not necessarily independent. These three effects may fortuitously add to result in an a_{XX} value of unity.

D. Results of Complexation of α-Cyclodextrin and Symmetrical 1,4-Disubstituted Benzene Compounds

Connors and Pendergast (28) examinined the complexation of α–cyclodextrin and symmetrical 1,4-disubstituted benzene compounds. The experimental binding constants K_{11} and K_{12} were determined and were interpreted using the binding model previously described. Table II shows these results for constants determined in 0.1 N NaCl aqueous solution at 25°C. Figure 3 shows the Hammett plot of the a_{XX} for these substrates. The slope is negative, as expected.

Table II

Stability Constants and Interaction Parameter for
α-Cyclodextrin Complexes with *sym* - X-C_6H_4-X at 25°C[a]

X	K_{11}/M^{-1}	K_{12}/M^{-1}	a_{XX}
NH_2 [b]	2.3		
OCH_3	75.4	221	11.7
OC_2H_5	128	326	10.2
I	5060	6250	4.94
Br	913	397	1.74
Cl	232	90	1.55
CO_2H	1344	23.8	0.071
CO_2CH_3	454	106	0.93
$COCH_3$	10.2		
CN	33.1	7.2	0.87
NO_2	35.8	4.6	0.51

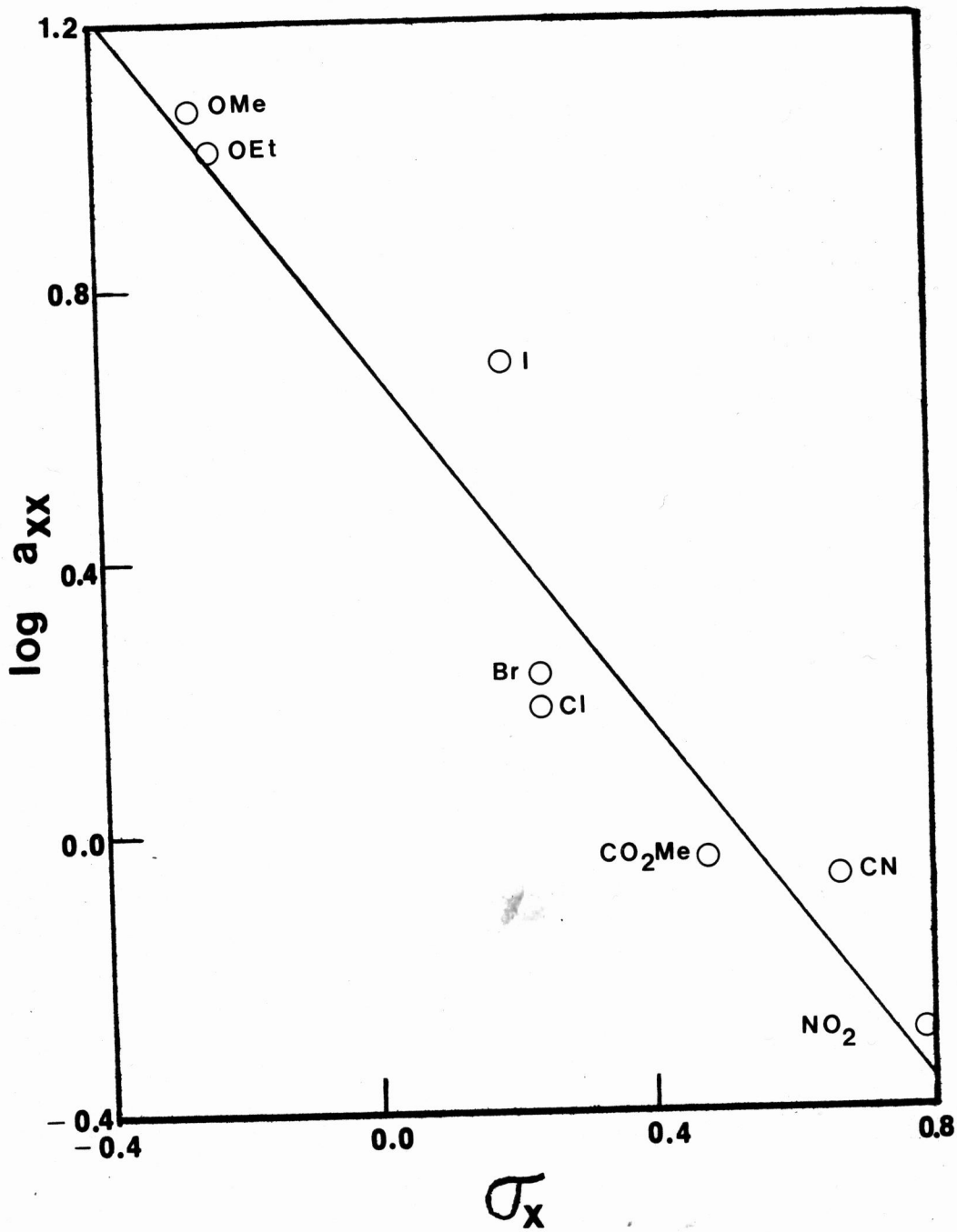
a ref. 28

b ref. 45

Figure 3

Hammett plot of a_{XX}, for *sym* - X- C_6H_4 -X. (Data from Table I)

Linear regression line shown.


1.2
0.8
0.4
0.0
−0.4
−0.4
0.0
0.4
0.8
log a_{xx}
σ_x
OMe
OEt
I
Br
Cl
CO_2Me
CN
NO_2

E. Research Plan

The Hammett plot of a_{XX} values for symmetrical two-site substrates has been shown to be linear (28). The a_{XX} term involves contributions by the repostitioning and ligand-ligand interaction effects, in addition to the electronic effect. We wish to isolate the electronic effect. Two structural features of the substrate seem to be important. (1) To maintain electronic transmission between sites, the substrate must be capable of resonance between the two binding sites. (2) For the repositioning and ligand-ligand interactions to be negligible, an adequate distance must separate the two binding sites. The required magnitude of this distance is not known at this time.

In this work, we experimentally determined the constants K_{11} and K_{12} for binding of α-cyclodextrin and a series of symmetrical 4,4'-disubstituted biphenyl compounds. These compounds were chosen because they are capable of resonance between sites, and because they provide an increased distance between sites, compared with the analogous benzene compounds studied by Pendergast.

We will apply the binding model for two site substrates described by Rosanske and Connors (29), and calculate the microscopic binding constants and a_{XX}. We will examine the Hammett plot of a_{XX} for biphenyl compound and compare it with the Hammett plot for the benzene compounds.

III EXPERIMENTAL METHODS

A Methods of Determining Binding Constants

Binding constants can be determined by observing a property of the substrate that changes upon complexation with ligand. The following are commonly used methods for studying binding equilibria of cyclodextrins in solution. Potentiometric methods are used to observe the change in apparent pKa of an ionizable substrate upon complexation (24, 30). For substrates in which only 1:1 binding occurs, the Benesi-Hildebrand (31) treatment can be applied to spectrophotometric (19) or fluorometric (19) changes in the substrate due to complexation. In competetive indicator methods (32, 33), the equilibrium between an absorbing indicator and cyclodextrin is perturbed by the addition of a non-absorbing substrate. The change in indicator binding is observed spectrophotometrically. In ^{1}H or ^{13}C nuclear magnetic resonance methods (23, 34), a change in chemical shift of the substrate or ligand is observed. In the solubility method (35) , an increase in the aqueous solubility of the substrate is observed upon complexation.

The solubility method was chosen for this work because it offered several advantages. First, several mathematical treatments of solubility data have been described in the literature. The constants for both 1:1 and 1:2 binding can be estimated. Also, the solubility method is suitable for compounds with low aqueous solubility. Substituted biphenyl compounds are relatively water insoluble compounds with two binding sites. Several of the other methods are not applicable because of the properties (very low solubility, non-ionizable nature) of the biphenyl substrates.

B Theory of the Solubility Method

The total aqueous solubility of a substrate will be increased upon complexation with cyclodextrin. In the solubility method, an aqueous solution of ligand, at concentrations below its solubility, and excess substrate is brought to solubility equilibrium. The solution phase is analyzed for total substrate concentration (S_t) as a function of total ligand concentration (L_t). A mathematical treatment for such experimental data was described by Higuchi and Kristiansen (36), and Kakemi and coworkers (37).

The equilibria are

$$S + L \rightleftharpoons SL$$

$$SL + L \rightleftharpoons SL_2$$

and the equilibrium constants are defined by

$$K_{11} = [SL] / [S] [L] \qquad (12)$$

$$K_{12} = [SL_2] / [SL] [L]. \qquad (13)$$

The mass balances on S_t and L_t are

$$S_t = [S] + [SL] + [SL_2] \qquad (14)$$

$$L_t = [L] + [SL] + 2[SL_2]. \qquad (15)$$

Excess substrate is always present, so that [S] is held at S_0, the substrate aqueous solubility. Therefore, eq. 14 becomes

$$S_t = S_0 + [SL] + [SL_2]. \qquad (16)$$

If we combine eqs. 12 through 16 to eliminate complex concentrations, the mass balance equations become

$$S_t = S_0 + K_{11}S_0[L] + K_{11}K_{12}S_0[L]^2 \qquad (17)$$

$$L_t = [L] + K_{11}S_0[L] + 2K_{11}K_{12}S_0[L]^2. \qquad (18)$$

If we solve eq. 18, which is quadratic in [L], we get eq. 19:

$$[L] = \frac{-(1 + K_{11}S_0) \pm \{(1 + K_{11}S_0)^2 + 8K_{11}K_{12}S_0L_t\}^{1/2}}{4K_{11}K_{12}S_0}$$

in which the positive root is the only real root. Moreover, eq. 17 can be rearranged to

$$(S_t - S_0) / [L] = K_{11}S_0 + K_{11}K_{12}S_0[L]\,. \qquad (20)$$

From a plot of $(S_t - S_0) / [L]$ versus $[L]$, we can obtain estimates of K_{11} and K_{12} from the slope and intercept. However, the experimental independent variable is

L_t, whereas eq. 20 is a function of [L]. We can calculate [L] at each L_t from eq. 19, then use that calculated [L] in the linear plot of eq. 20. We use the estimated K_{11} and K_{12} values from this plot in eq. 19 to recalculate [L], and use that [L] again in eq. 20 to estimate improved values of K_{11} and K_{12}. The iteration continues until values of K_{11} and K_{12} converge.

The values of K_{11} and K_{12} are considered convergent when the leading digit that changes is the fifth significant figure. This generally occurs in seven to nine iterations.

A first estimate of K_{11} and K_{12} is needed in eq. 19 to begin the iteration. To obtain this, we make the approximation in eq. 20 that [L] = L_t. From a plot of $(S_t - S_0) / L_t$ versus L_t, we get acceptable first estimates from the slope and intercept.

C Data Treatment

Least squares analysis was applied to the experimental data to estimate K_{11}, K_{12}, a_{xx}, and their standard deviations. The data were plotted according to eq. 20. Because of the linearizing transforms required to reach eq. 20, it must be determined whether weighted or unweighted least squares analysis is appropriate for this system. Unweighted least squares analysis is valid if the variance of the ordinate is constant. Preliminary experiments were performed with 1,4 - dimethylterephthalate as a substrate. S_t was determined in triplicate over a range of thirteen L_t values. When the relative

variance of the ordinate was plotted as a function of the value of the abcissa, no correlation was observed, i.e. the relative variance of the of the ordinate is independent of the abscissa value. Unweighted least squares analysis is therefore applicable. The variance of the parameters was then calculated through a propagation of errors treatment, according to the general eq. 21,

$$\sigma_Y^2 = (\partial F/\partial X_1)^2 \sigma_{X_1}^2 + (\partial F/\partial X_2)^2 \sigma_{X_2}^2 + \ldots \qquad (21)$$

where $Y = F(X_1, X_2, \ldots)$. Specifically, the variances of K_{11}, K_{12}, and a_{XX} are given by eqs. 22, 23, and 24,

$$\sigma_{K_{11}}^2 = \frac{\sigma_{S_o}^2}{S_o^2} K_{11}^2 + \frac{\sigma_b^2}{b^2} K_{11}^2 \qquad (22)$$

$$\sigma_{K_{12}}^2 = \frac{\sigma_b^2}{b^2} K_{12}^2 + \frac{\sigma_m^2}{m^2} K_{12}^2 \qquad (23)$$

$$\sigma_{a_{XX}}^2 = \frac{\sigma_{K_{11}}^2}{K_{11}^2} a_{XX}^2 + \frac{\sigma_{K_{12}}^2}{K_{12}^2} a_{XX}^2 \qquad (24)$$

where m and b represent the slope and intercept , respectively, of eq. 20.

The least squares analysis of the data plotted according to eq. 20 was performed by computer. This computer program (BASIC language) is reproduced in appendix A. Appendix B contains the program which calculates the first estimates of

K_{11} and K_{12}, which are needed to begin the iteration.

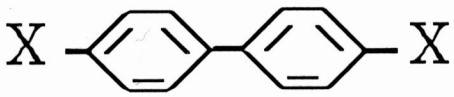
D Materials and Equipment

α-Cyclodextrin (Sigma) was dried at 105°C for at least three hours and was weighed immediately. Lin (38) observed no difference between results from recrystallized and unrecrystallized α-cyclodextrin obtained from Sigma. Therefore, recrystallization of the ligand was regarded as unnecessary. Solvents used in the study were deionized distilled water, which was redistilled from alkaline permanganate, and anhydrous methanol (Mallinckrodt or Fischer).

All substrates were recrystallized from methanol. Table III shows their manufacturers and their experimental and literature melting points. Spectrophotometric measurements were made with either a Perkin-Elmer Model 559 or a Cary-Varian Model 2200 UV-Visible Recording Spectrophotometer. The thermostated cell compartments of these instruments were connected to external water baths capable of maintaining a temperature of 25 ± 0.1 °C.

TABLE III

Identification of Substrate Materials



X	Source	Observed melting point (°C)	Literature melting point (°C)	Reference
-H	Aldrich	68-69	70.5	39
$-CH_3$	Aldrich	119	120.7-121.5	40
-Cl	Pfaltz & Bauer	148	148-149	41
-OH	Tokyo Kasei Kogyo	280-281	286	42
			280.5	43

E. Experimental Procedures

α-Cyclodextrin was dried for at least three hours at 105 °C to give the anhydrous powder, which was weighed immediately to prepare stock solutions. The solvent for all studies was 0.1 N NaCl aqueous solution. From the stock ligand solution, dilutions were made to produce solutions with a range of ligand concentrations.

An excess of solid substrate was placed in 4 dram glass screwcap vials, and approximately 8.5 ml. of α-cyclodextrin solution of differing concentrations was added. To determine S_0, excess substrate was placed in vials and approximately 10 ml. of 0.1 N NaCl aqueous solution was added. All vials were sealed with Teflon® lined screw caps and placed in a water bath at 25 ± 0.1 °C. The vials were rotated end over end for at least 24 hours. Pendergast (44) determined that 24 hours was adequate equilibration time for substances with a wide range of solubilities, so 24 hours was taken as the minimum equilibration time.

After equilibration, the vials were removed and the solid phase separated from the solution phase by filtration through a 0.22 μ Teflon® coated filter supported by a 25 mm Millipore Swag-Loc filter apparatus.

For the solutions containing ligand, a measured volume was immediately withdrawn and diluted such that the final analytical solvent composition was 1 part water to 4 parts methanol. This solvent served to dissociate the complex. The total substrate concentration of these solutions was then determined spectrophotometrically. The following preliminary experiment validated that complete dissociation of the complex occurred when the solution phase was diluted with methanol. Ultraviolet spectra of

substrate solutions in 4:1 methanol:water, 0.1 N NaCl, were compared with spectra of substrate solutions at the same concentration in the same solvent, but with ligand added. The appropriate reference solutions for this experiment were solvent without and with ligand, respectively. Biphenyl, 4,4'-dihydroxybiphenyl and 4,4'-dichlorobiphenyl showed no spectral differences. The dimethyl substituted compound showed a small shift, but calculations showed that the estimates of the binding constants were not affected significantly because of this shift.

For the solutions in the absence of ligand, the more soluble biphenyl and dihydroxy-substituted compound were diluted to produce a 1:1 methanol:water, 0.1 N NaCl solution. The substrate solubility was determined spectrophotometrically in 1 cm cells. The reference solution was 1:1 methanol:water, 0.1 N NaCl.

The dichoro- and dimethyl- substituted compounds had very low solubilities and dilution caused the analytical solution to be below the range of detection. Therefore, the substrate solubility was determined from undiluted solution phase. The concentration was determined spectrophotometrically in 10 cm cells. The filtration step occurred only seconds prior to the determination of absorbance. The reference cell contained 0.1 N NaCl aqueous solution.

All substrate concentrations were determined at the wavelength of maximum absorption. Beer's law plots were made for each compound and were found to be linear. Table IV gives a summary of the spectral properties of the substrates.

Table IV

Spectral Properties of *sym* -X-C_6H_4 - C_6H_4 -X

X	ε_{max} / 10^4 M^{-1} cm^{-1}	λ_{max} / nm
-OH	2.06	261.5
$-CH_3$	2.28	254.0
-H	1.67	247.5
-Cl	2.24	259.5

IV RESULTS

The data from the solubility experiments on symmetrical disubstituted biphenyl compounds are reported in this chapter. In Tables V through VIII, these data are shown. Columns three and four of these tables are the ordinate and abscissa, respectively, of the linear equation, eq. 20. S_o, K_{11}, K_{12}, and a_{XX} are also shown, with their standard deviations in parentheses. In Figures 4, 6, 8, and 10, the raw data is shown graphically. The curves drawn are the calculated curves from eq. 17. The [L] values used in eq. 17 are calculated from eq. 19, using the final estimates of the binding constants K_{11} and K_{12}. Figures 5, 7, 9, and 11 show the data plotted according to the linear equation, eq. 20.

In the case of 4,4'-dimethylbiphenyl multiple S_t values were determined at a single L_t value, for two L_t values. At $L_t = 1 \times 10^{-2}$ M, 4 S_t values were determined. At $L_t = 1 \times 10^{-1}$ M, 5 S_t values were determined. In Figure 10 , the mean S_t values at those L_t values are shown. At $L_t = 1 \times 10^{-2}$, one standard deviation is approximately $0.08 \cdot r$, where r is the radius of the circle shown. At $L_t = 1 \times 10^{-1}$, one standard deviation is approximately $0.6 \cdot r$. In Figure 11, the mean $(S_t - S_o) / [L]$ value is shown for both [L] values, and one standard deviation is indicated by a bar.

Table IX shows the relative standard deviations of the two ordinate values at the two [L] values for 4,4' - dimethylbiphenyl. The relative standard deviations are quite close for [L] values an order of magnitude apart. This is evidence that unweighted least squares analysis of eq. 20 is applicable. This evidence is in addition to data from the preliminary experiment performed on dimethylterephthalate, which was previously described in the data treatment section of this work.

Table V

Solubility data for Biphenyl in 0.1 N NaCl at 25°C.

$L_t / 10^{-2}$ M	$S_t / 10^{-5}$ M	[L] / 10^{-2} M	$\frac{S_t - S_o}{[L]} / 10^{-3}$
0.200	4.43	0.199	2.14
0.400	5.00	0.399	2.52
0.600	5.64	0.598	2.75
0.800	6.51	0.797	3.15
1.00	7.03	0.995	3.04
1.29	9.05	1.28	3.94
2.58	17.4	2.56	5.22
3.87	30.7	3.83	6.98
5.16	46.5	5.09	8.35
6.45	67.6	5.34	10.0

$S_o = 4.0 \times 10^{-5} (0.2 \times 10^{-5})$ M

$K_{11} = 50 (3.2)$ M^{-1}

$K_{12} = 63 (2.6)$ M^{-1}

$a_{XX} = 5.0 (0.4)$

Table VI

Solubility data for 4,4' - dihydroxybiphenyl in 0.1 N NaCl at 25°C.

$L_t / 10^{-2}$ M	$S_t / 10^{-4}$ M	[L] / 10^{-2} M	$\frac{S_t - S_o}{[L]} / 10^{-2}$
0.319	2.53	0.311	1.79
0.425	2.74	0.412	1.85
0.531	3.13	0.512	2.24
0.637	3.51	0.611	2.51
0.743	4.02	0.709	2.88
0.850	4.39	0.806	2.99
0.854	4.62	0.810	3.26
0.956	4.94	0.902	3.28
1.06	5.74	0.998	3.77
1.28	6.86	1.19	4.10

$S_o = 1.98 \times 10^{-4} (0.01 \times 10^{-4})$ M

$K_{11} = 41 (5)\ M^{-1}$

$K_{12} = 345 (48)\ M^{-1}$

$a_{XX} = 33 (6)$

Table VII

Solubility data for 4,4' - dichlorobiphenyl in 0.1 N NaCl at 25°C.

$L_t / 10^{-2}$ M	$S_t / 10^{-5}$ M	[L] / 10^{-2} M	$\frac{S_t - S_o}{[L]} / 10^{-3}$
0.202	0.281	0.2018	1.26
0.303	0.496	0.3025	1.55
0.405	0.796	0.4031	1.91
0.506	1.27	0.5034	2.47
0.607	1.80	0.6036	2.94
0.809	2.82	0.8034	3.47
1.01	4.81	1.002	4.78
1.24	7.30	1.227	5.93
1.51	9.86	1.490	6.60
2.04	17.9	2.004	8.93
2.48	26.5	2.432	10.9

$S_o = 2.6 \times 10^{-7}$ (0.4×10^{-7}) M

$K_{11} = 1030$ (390) M^{-1}

$K_{12} = 1600$ (553) M^{-1}

$a_{XX} = 6$ (3.2)

Table VIII

Solubility data for 4,4' - dimethylbiphenyl in 0.1 N NaCl at 25°C.

$L_t / 10^{-2}$ M	$S_t / 10^{-5}$ M	[L] / 10^{-2} M	$\frac{S_t - S_o}{[L]} / 10^{-3}$
0.212	0.261	0.212	0.973
0.425	0.416	0.424	0.852
0.637	0.632	0.636	0.908
0.850	1.09	0.848	1.23
1.062	1.40	1.060	1.27
1.062	1.38	1.060	1.25
1.602	1.37	1.060	1.24
1.062	1.29	1.060	1.16
2.08	4.06	2.07	1.94
3.11	7.95	3.10	2.55
4.15	12.8	4.13	3.09
5.19	17.6	5.15	3.41
7.27	41.3	7.19	5.73
9.34	61.8	9.22	6.69
10.4	77.1	10.2	7.53
10.4	76.3	10.2	7.45
10.4	74.1	10.2	7.23
10.4	72.1	10.2	7.04
10.4	78.2	10.2	7.63

Table VIII (continued)

Solubility data for 4,4' - dimethylbiphenyl in 0.1 N NaCl at 25°C.

$S_o = 5.4 \times 10^{-7} (0.5 \times 10^{-7})$ M

$K_{11} = 1000 (175)$ M^{-1}

$K_{12} = 120 (18)$ M^{-1}

$a_{XX} = 0.5 (0.1)$

Figure 4 Plot of solubility data (Table V) for biphenyl. The curve shown is calculated from eq. 17.

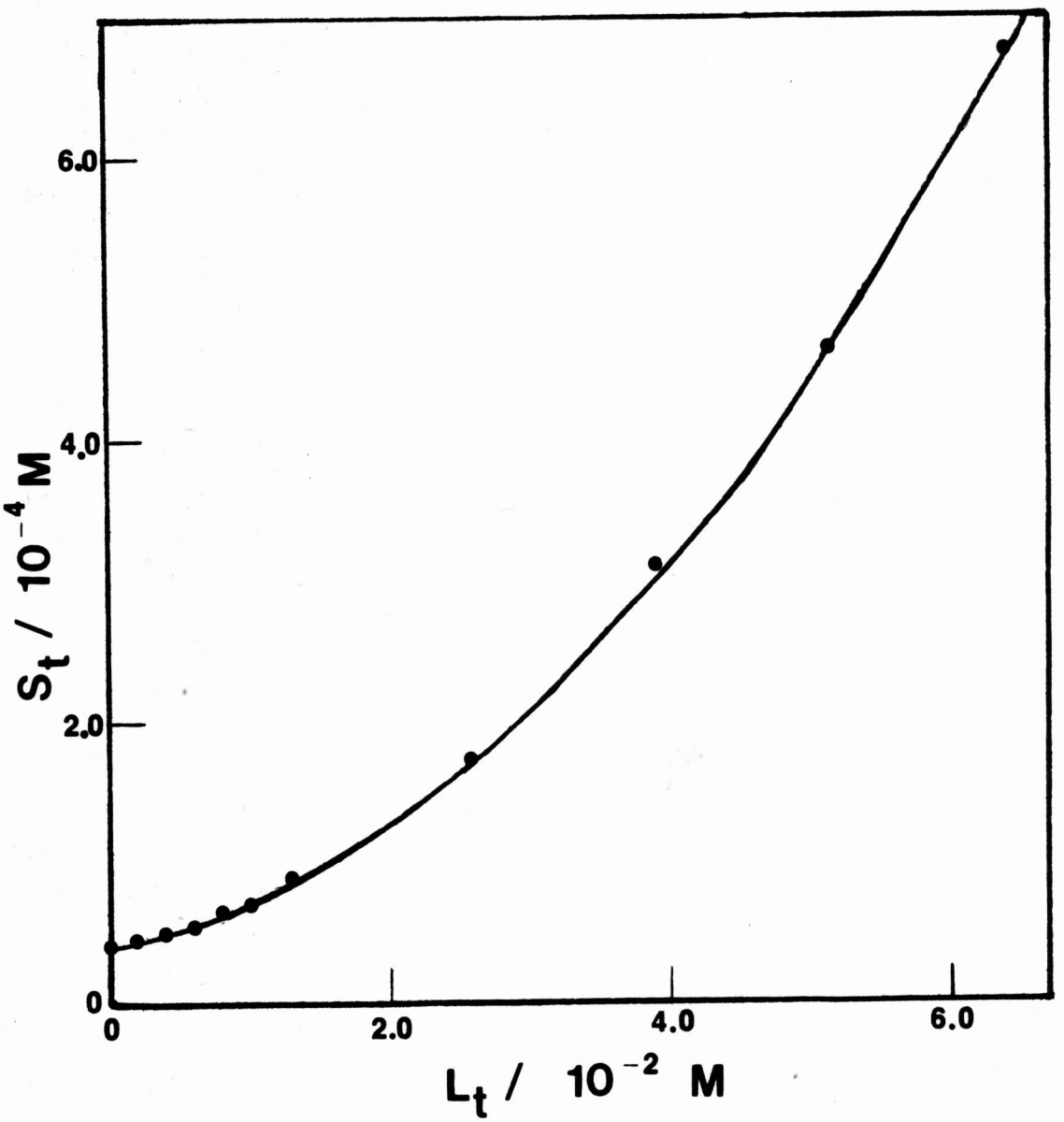

6.0
4.0
2.0
0
0
2.0
4.0
6.0
S_t / 10^{-4} M
L_t / 10^{-2} M

Figure 5 Plot of solubility data (Table V) for biphenyl, according to eq. 20. Linear regression line is shown.

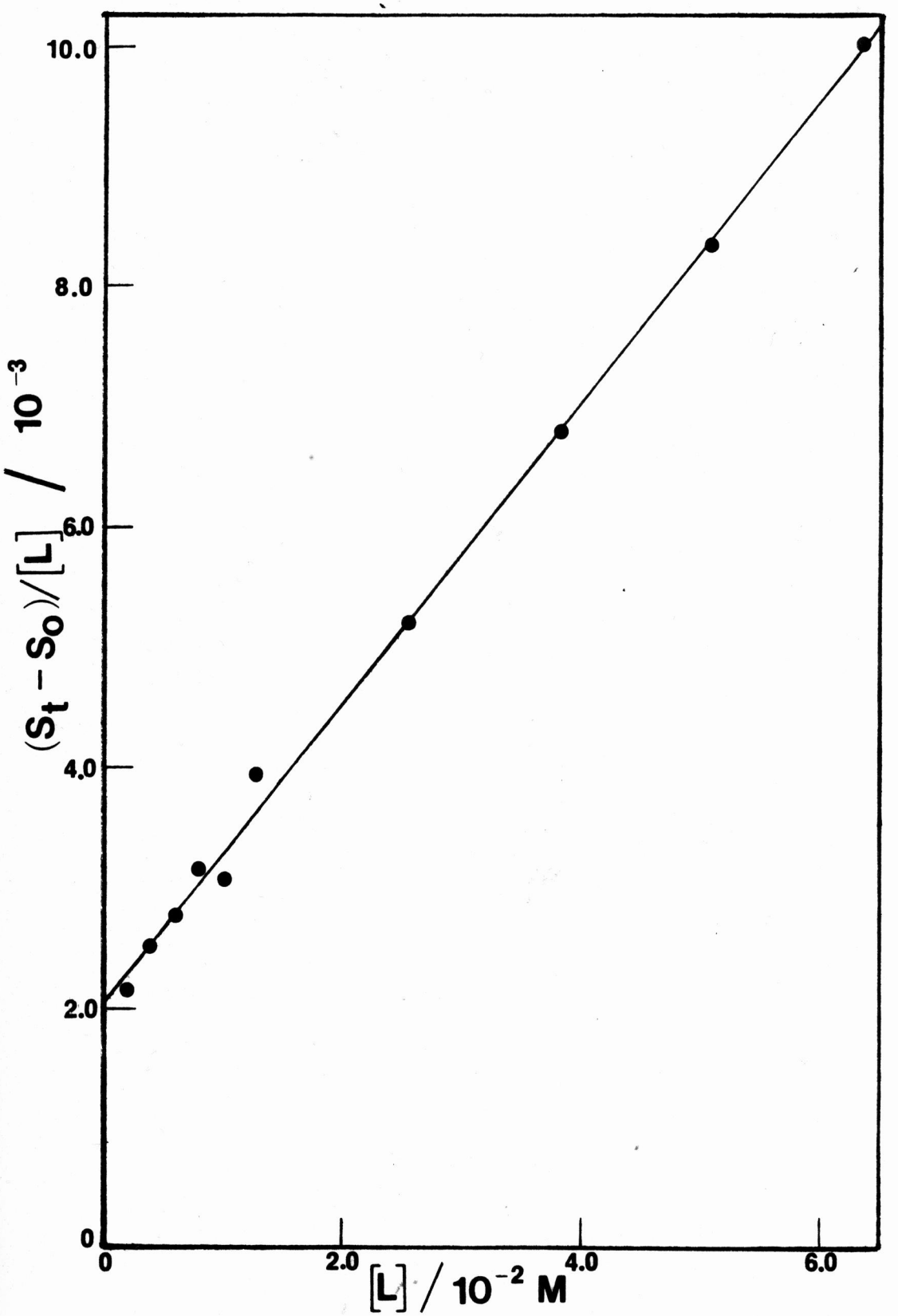

10.0
8.0
6.0
4.0
2.0
0
0
2.0
4.0
6.0
$(S_t - S_o)/[L] / 10^{-3}$
$[L] / 10^{-2}$ M

Figure 6 Plot of solubility data (Table VI) for 4,4' - dihydroxybiphenyl. The curve shown is calculated from eq. 17.

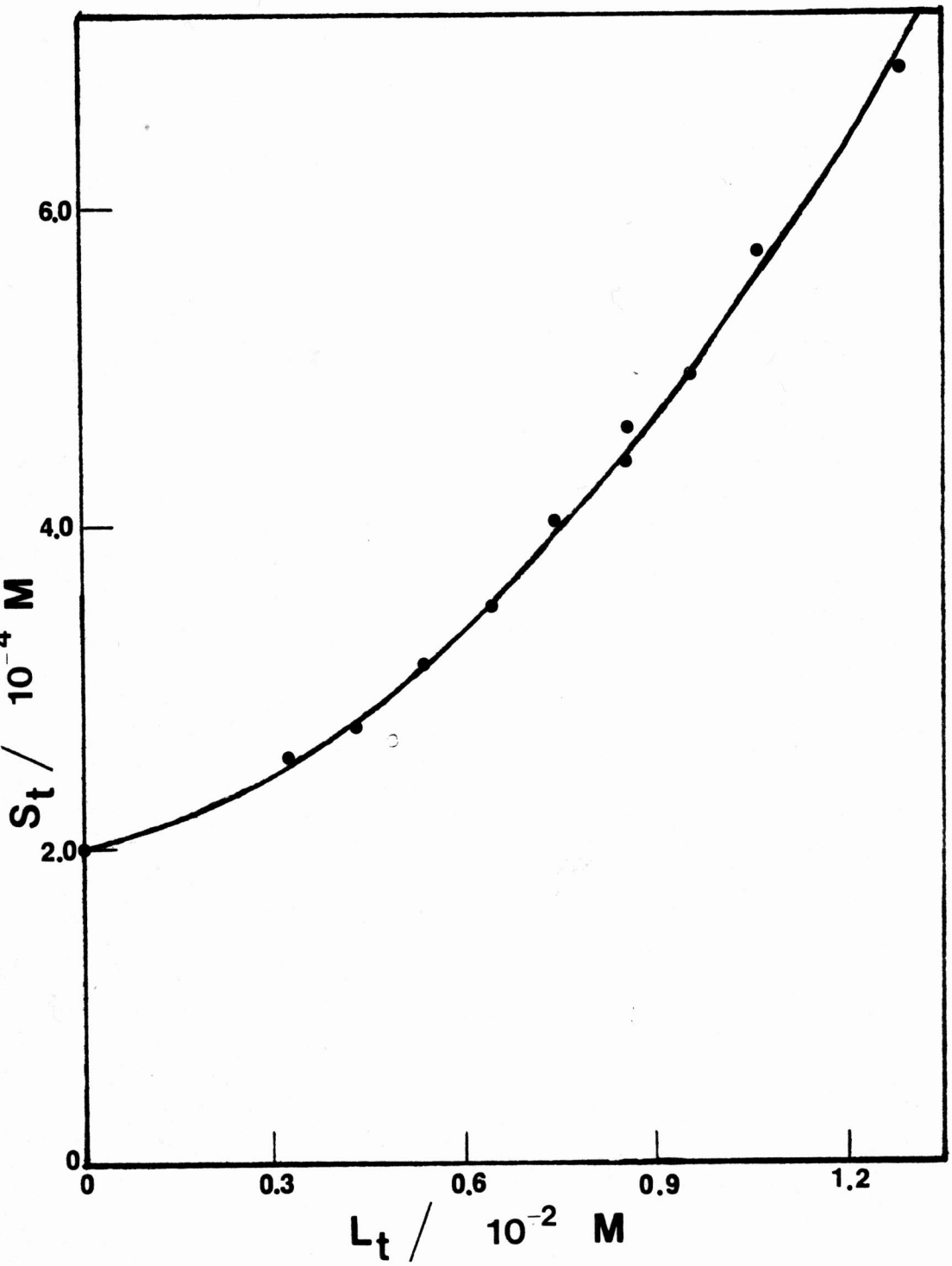

6.0
4.0
2.0
0
S_t / 10^{-4} M
0
0.3
0.6
0.9
1.2
L_t / 10^{-2} M

Figure 7 Plot of solubility data (Table VI) for 4,4' - dihydroxybiphenyl, according to eq. 20. Linear regression line is shown.

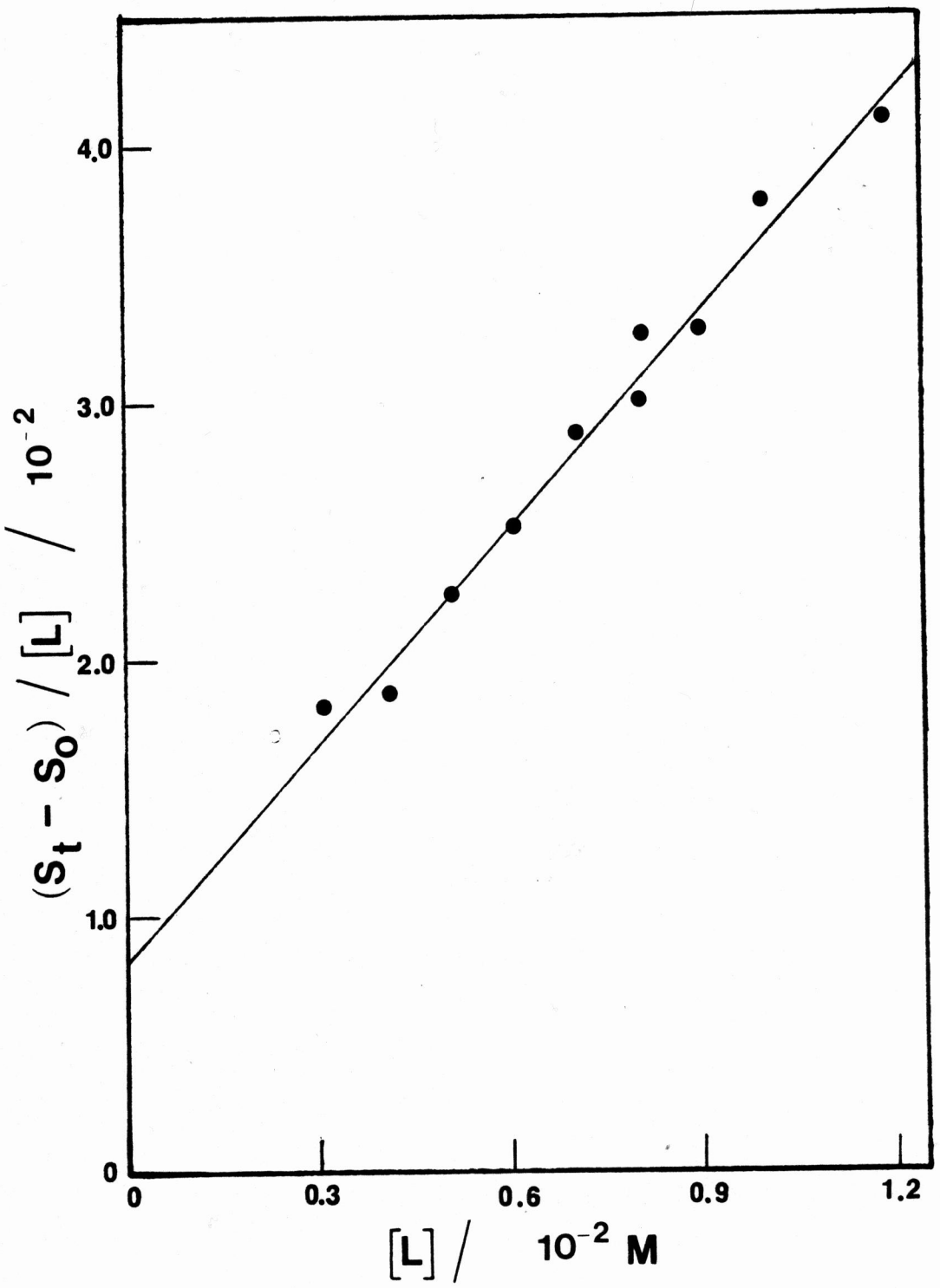

4.0
3.0
2.0
1.0
0
0
0.3
0.6
0.9
1.2
$(S_t - S_o) / [L] / 10^{-2}$
$[L] / 10^{-2}$ M

Figure 8 Plot of solubility data (Table VII) for 4,4' - dichlorobiphenyl. The curve shown is calculated from eq. 17.

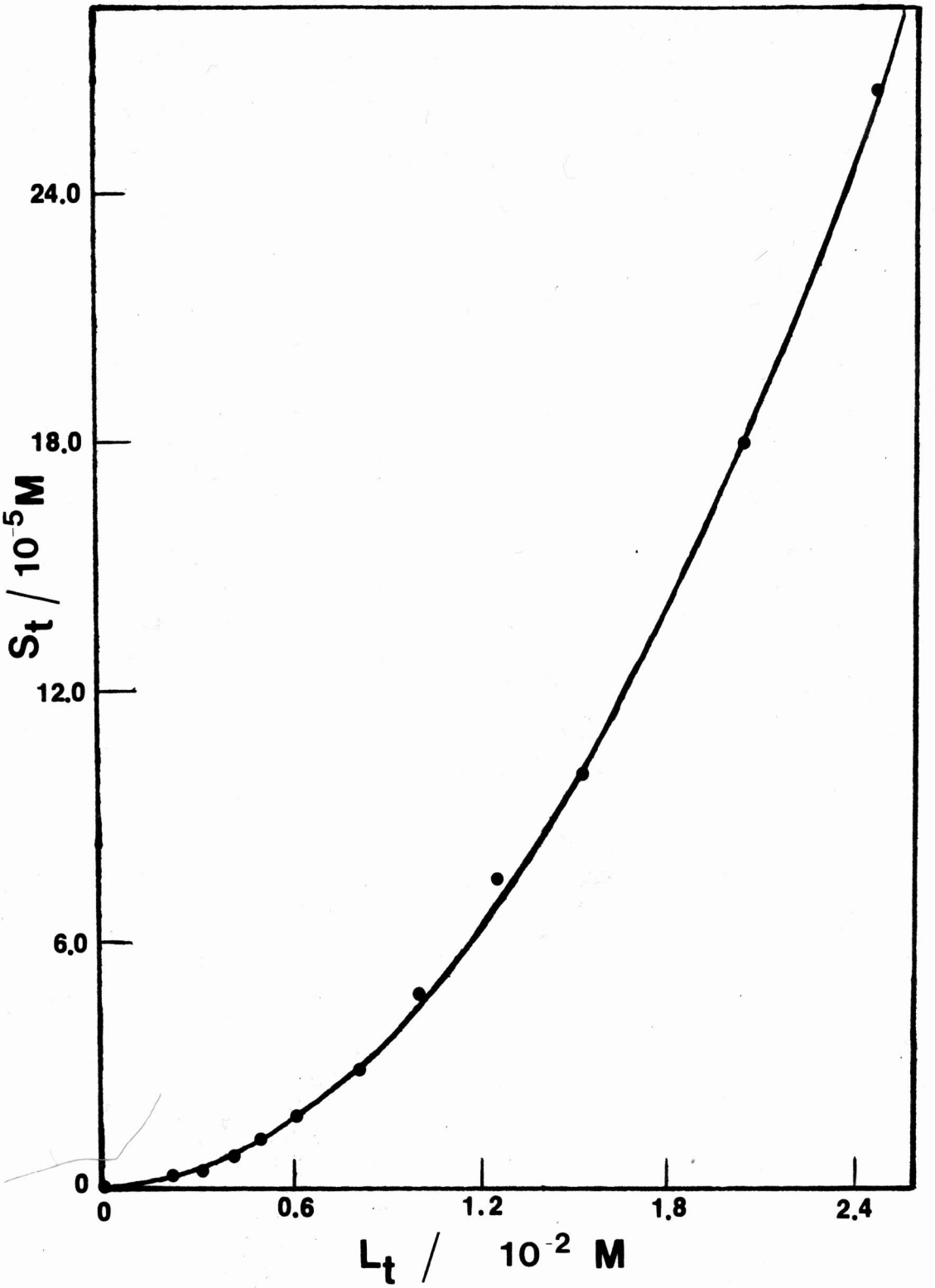

24.0
18.0
12.0
6.0
0
S_t / 10^{-5} M
0
0.6
1.2
1.8
2.4
L_t / 10^{-2} M

Figure 9 Plot of solubility data (Table VII) for 4,4' - dichlorobiphenyl according to eq. 20. Linear regression line is shown.

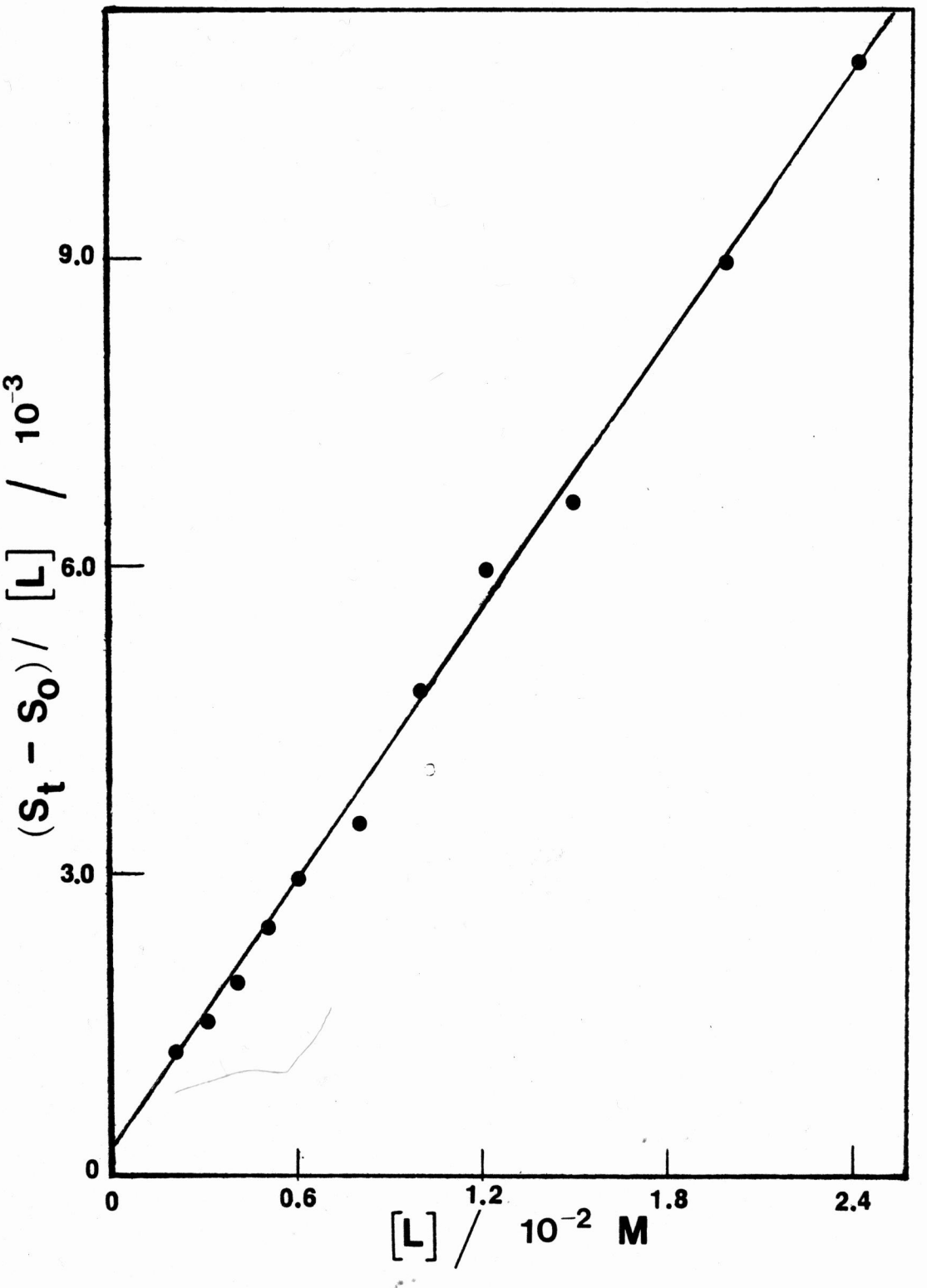

9.0
6.0
3.0
0
$(S_t - S_o) / [L] / 10^{-3}$
0
0.6
1.2
1.8
2.4
$[L] / 10^{-2}$ M

Figure 10 Plot of solubility data (Table VIII) for 4,4' - dimethylbiphenyl. The curve shown is calculated from eq. 17.

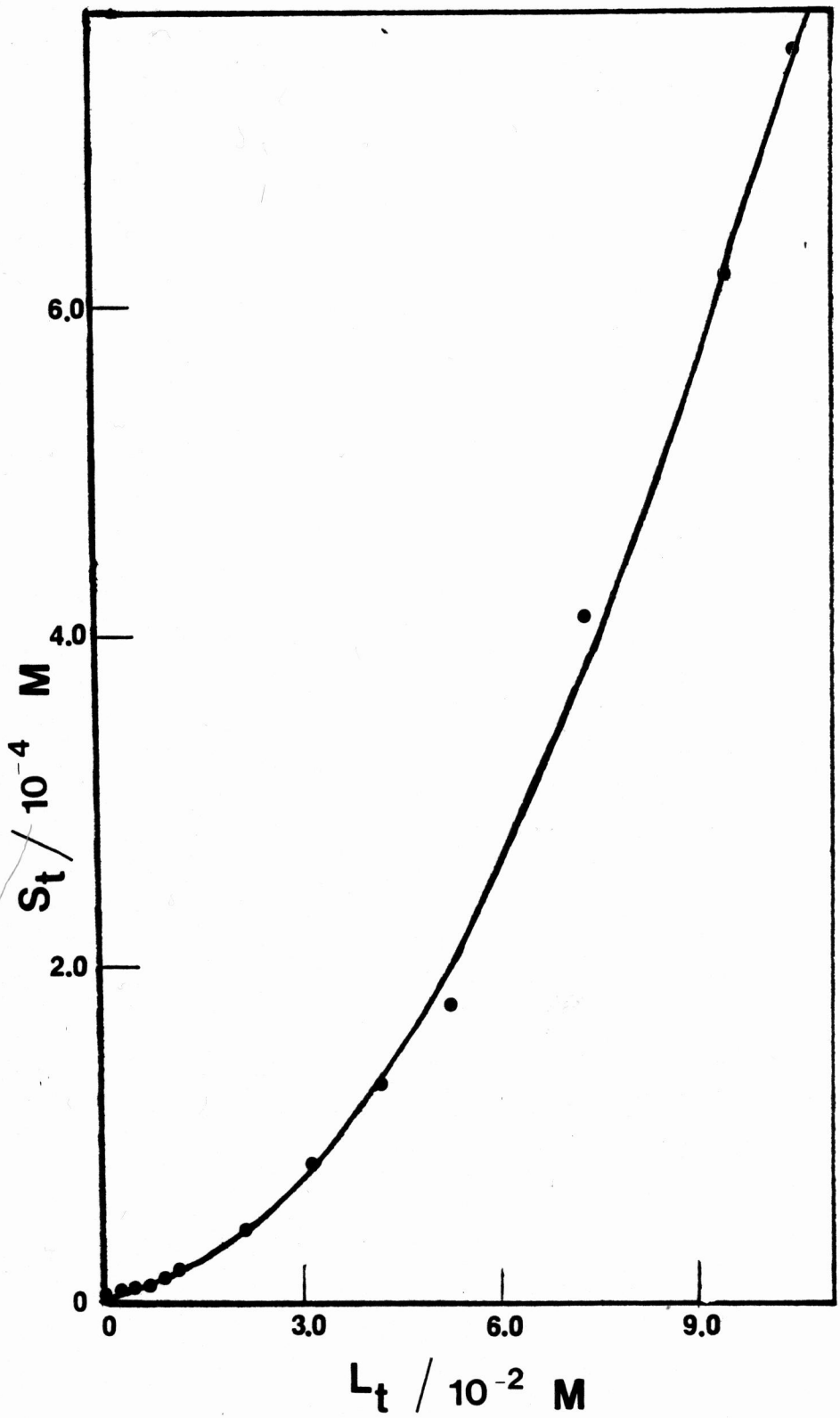

6.0
4.0
2.0
0
S_t / 10^{-4} M
0
3.0
6.0
9.0
L_t / 10^{-2} M

Figure 11 Plot of solubility data (Table VIII) for 4,4' - dimethylbiphenyl according to eq. 20. Linear regression line is shown.

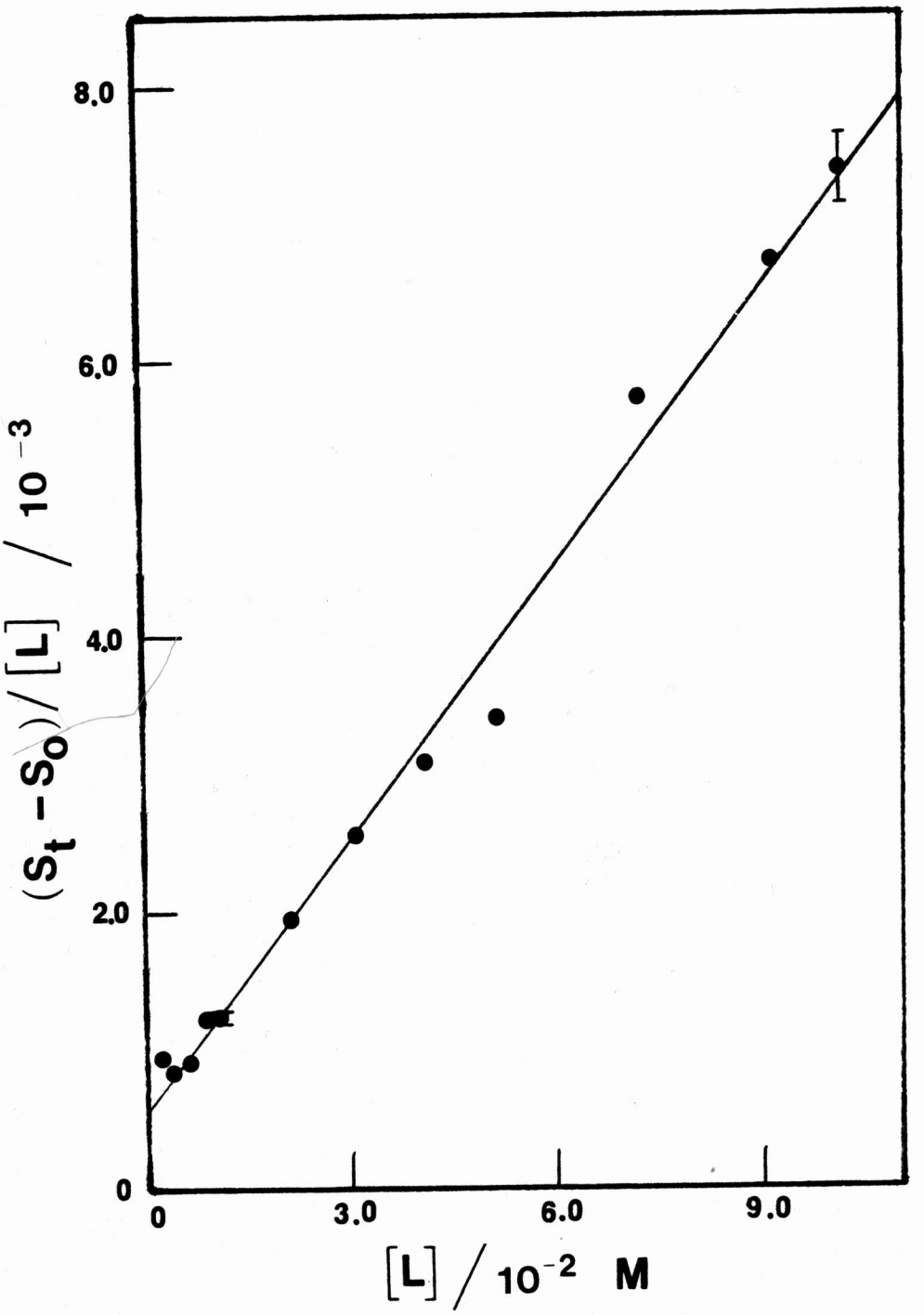

8.0
6.0
4.0
2.0
0
0
3.0
6.0
9.0
$(S_t - S_o)/[L] \ / \ 10^{-3}$
$[L] \ / \ 10^{-2}$ M

Table IX

Relative standard deviations of $(S_t - S_o) / [L]$ for 4,4' - dimethylbiphenyl

$L_t / 10^{-2}$ M	$[L] / 10^{-2}$ M	relative standard deviation of $(S_t - S_o) / [L]$
1.062	1.060	3.66×10^{-2}
10.38	10.24	3.25×10^{-2}

Table X is a summary of the results of the solubility studies. In addition to the constants determined in this work, results of solubility studies on four additional symmetrical 4,4-disubstituted biphenyl compounds are included. This additional work was performed by D. Toledo-Velasquez (47).

Table X Constants for binding of α-cyclodextrin and *sym* - X- C_6H_4 - C_6H_4 - X[a]

X	$S_o/10^{-5}$ (M)	$K_{11}/(M^{-1})$	$K_{12}/(M^{-1})$	a_{XX}
OH	19.8 (0.1)	41 (5)	345 (48)	33 (6)
CH_3	0.054 (0.005)	1000 (175)	123 (18)	0.5 (0.1)
H	4.0 (0.2)	50 (3)	63 (3)	5.0 (0.4)
Cl	0.026 (0.004)	1030 (400)	1620 (550)	6.3 (3.2)
Br[b]	0.00625 (0.0021)	4330 (1985)	5330 (2173)	4.9 (2.9)
COOH[b]	0.22 (0.03)	14300 (3850)	845 (210)	0.23 (0.08)
CN[b]	0.52 (0.07)	220 (35)	66 (20)	1.2 (0.4)
NO_2[b]	0.047 (0.004)	860 (100)	147 (17)	0.7 (0.1)

a standard deviations in parentheses.

b ref. 47

V DISCUSSION

A. Discussion of a_{XX} for the Biphenyl Series

The discussion of the data presented in this work will concentrate on the interaction parameter, a_{XX}. In the section of this work entitled " Effects on the Magnitude of a_{XX}," the electronic effect was discussed. From that discussion, one would expect to see a correlation between a_{XX} and σ_X, the Hammett substituent constant, due to the electronic effect. Pendergast (28) found a good linear correlation ($r = 0.96$) between a_{XX} and σ_X for eight of nine symmetrical 1,4-disubstituted benzene compounds (see Fig. 3.) Terephthalic acid showed a large negative deviation, and does not appear in Fig. 3.

Table XI lists the experimental estimates of a_{XX} and literature σ_X for symmetrical 4,4' - disubstituted biphenyl compounds. Figure 12 shows the Hammett plot for the biphenyl series. The dicarboxylic acid is a major negative deviator, as it was in the benzene series, and is not shown in Fig. 12. The dimethyl substituted biphenyl is also a negative deviator in the biphenyl series, and is shown in Fig. 12. In the biphenyl series, then, six of eight compounds are well correlated ($r = 0.97$). The Hammett plot shows a negative slope, as expected for the electronic effect.

Table XI

Interaction parameter for *sym* - X - C_6H_4 - C_6H_4 - X

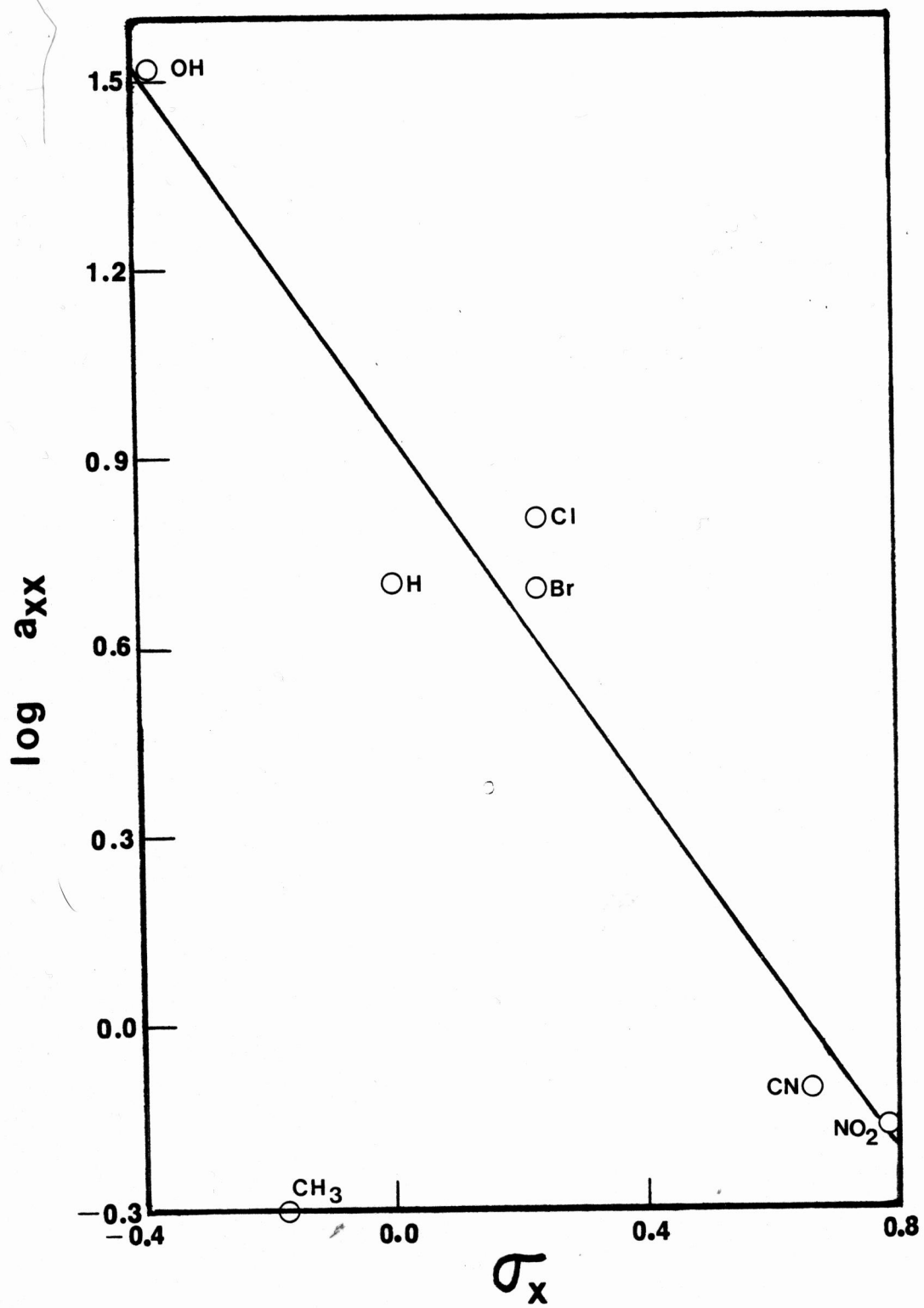
X	log a_{XX}	σ_X
-OH	+1.518	-0.370
$-CH_3$	-0.301	-0.170
-H	+0.699	0.000
-Cl	+0.799	+0.227
-Br[a]	+0.690	+0.232
-COOH[a]	-1.470	+0.450
-CN[a]	-0.108	+0.660
$-NO_2$[a]	-0.167	+0.778

a ref. 47

Figure 12

Hammett plot of a_{XX} (Table XI), for *sym* - X - C_6H_4 - C_6H_4 - X.

Linear regression line shown.


OH
1.5
1.2
0.9
Cl
H
Br
0.6
0.3
0.0
CN
NO2
CH3
−0.3
−0.4
0.0
0.4
0.8
log axx
σx

B. Discussion of Anomalous Substituents

The dicarboxylic acid substituted compound is a significant negative outlier in both series. The dimethyl substituted compound is a signifigant negative outlier in the biphenyl series, and was not examined in the benzene series. These same two substituents have been shown to be anomalous in other studies. Wong, Lin, and Connors (45) examined the complexation of α-cyclodextrin and a series of 4-substituted anilines at 25°. Table XII lists the K_{11} values obtained for the complexation of the conjugate base form of the aniline. The compounds are listed in order of increasing Hammett substituent parameter value. In examining the rank order in this series, one observes that the 4 - methylaniline and the 4 - aminobenzoic acid are anomalously large. Moreover,the data from this series were analyzed by multiple linear regression. Log K_{11} was found to be linearly dependent on two factors, σ_X and R_D, the molar refraction of $X\text{-}C_6H_4$. Again, the 4-carboxy- and 4-methyl- substituted compounds were positive deviators from the linear relationship. (Large K_{11} values correspond to small a_{XX} values.)

What factors could lead to deviations from the Hammett correlation for a_{XX} ? The Hammett parameter describes a substituent's electronic donating or withdrawing capability. From eq. 11, we see that a_{XX} is composed of contributions from the electronic effect, plus the repositioning and ligand-ligand interaction effects. The negative deviators in the benzene and biphenyl Hammett plots are a reflection of the

Table XII

Stability constants for α-cyclodextrin complexes of 4-substituted anilines at 25°C

X[a]	K_{11} / M^{-1}
$-NH_2$[b]	2.6
$-OCH_3$[b]	6.7
$-CH_3$[b]	57.6
-H[b]	8.8
$-COO^-$[c]	9.0
-Cl[b]	251
-COOH[c]	1341
-CN[b]	451
$-NO_2$[b]	635

a X in X - C_6H_4 - NH_2

b ref. 45

c ref. 24

additive components of a_{xx}. Because the deviations are negative, they indicate that the compounds involved exhibit a much larger repositioning effect than the other members of the series. Excess ligand-ligand interactions could only result in positive deviations.

A large repositioning effect would result from the substrate being more deeply inserted into the cyclodextrin cavity than the other members of the series. Formation of the 1:2 complexes in these cases would greatly perturb the 1:1 equilibrium positions and would thus make 1:2 complexation less favorable. Deep insertion into the cavity for the two anomalous compounds is consistent with the evidence in the aniline series of anomalously large K_{11} values for X = COOH and X = CH_3. The carboxy- substituted compounds are capable of hydrogen bonding, possibly with the primary hydroxyl groups on the more distant rim. No such easily identifiable chemical feature is present in the methyl-substituted compound.

C. Comparison of Hammett Correlations for the Benzene and Biphenyl Series

The Hammett plot of a_{XX} for the benzene series can be described by eq. 25 (ref. 28).

$$\log a_{XX} = -1.29\ (0.16)\ \sigma_X + 0.68\ (0.07) \qquad (25)$$

Standard deviations of the slope and intercept are shown in parentheses. The relationship for the biphenyl series is given by eq. 26. In both eqs. 25 and 26, the

$$\log a_{XX} = -1.45\ (0.18)\ \sigma_X + 0.94\ (0.08) \qquad (26)$$

a_{XX} values for the major deviators in each series (X = COOH, CH_3) were excluded from the least squares analysis.

The slope of a Hammett plot is termed the Hammett ρ value. At a 95% confidence level, the Hammet ρ values for the benzene and biphenyl series are not significantly different. Figure 13 shows the Hammett relationship for both series plotted on the same graph.

In previous discussion, we saw that the Hammett ρ value describes the effectiveness of the transmission of the electronic effect. Since the ρ values for the two series are not statistically different, we conclude that the transmission of the electronic effect is equally effective in the biphenyl series as in the benzene series.

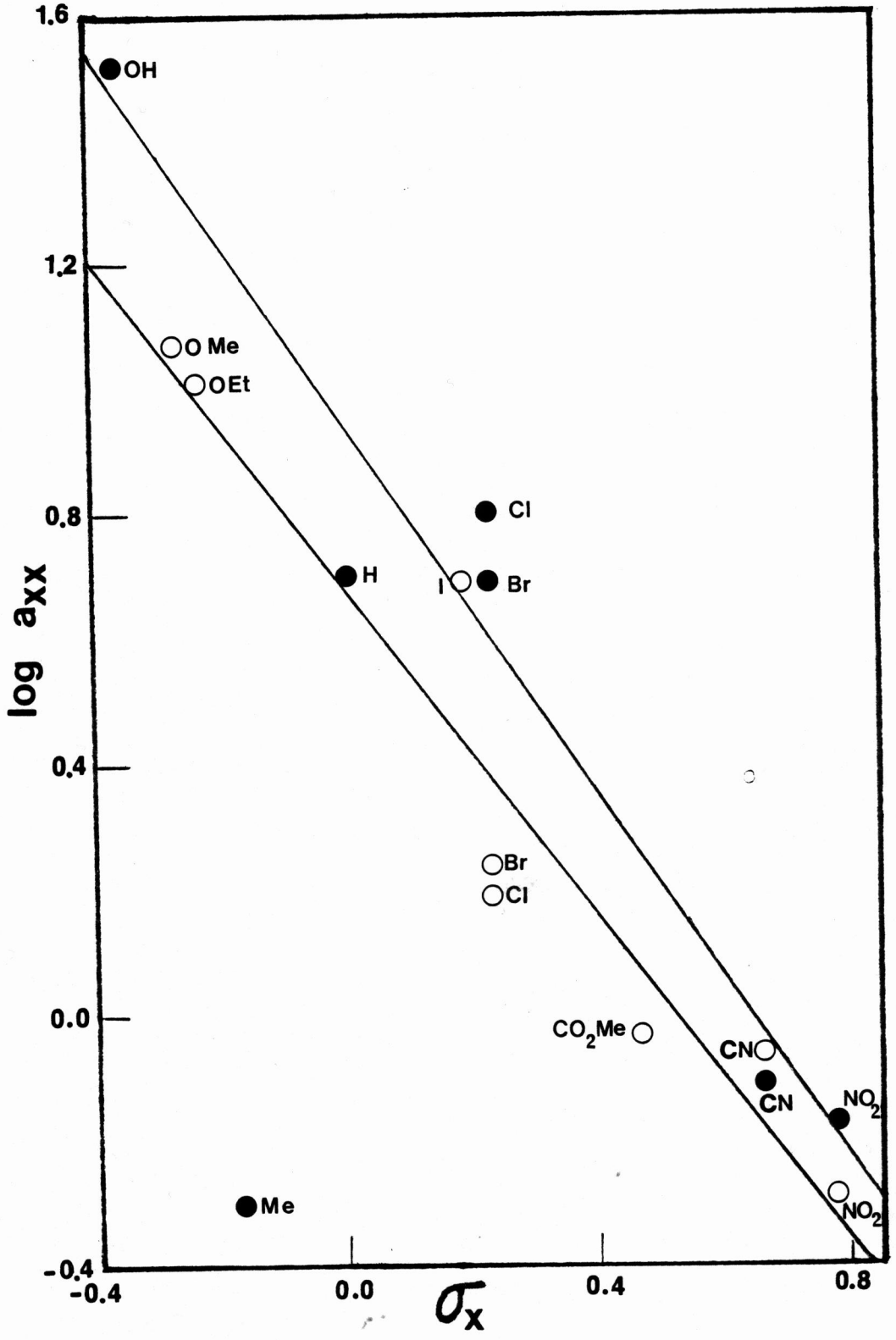
Figure 13

Hammett plot of a_{XX}

for X - C_6H_4 - X, open circles. (Data from Table II.)

for X - C_6H_4 - C_6H_4 - X, closed circles. (Data from Table XI.)

Linear regression lines shown.


1.6
1.2
0.8
0.4
0.0
-0.4
log a_{xx}
OH
O Me
OEt
Cl
H
I
Br
Br
Cl
CO_2Me
CN
CN
NO_2
NO_2
Me
-0.4
0.0
0.4
0.8
σ_x

D. Discussion of the Dihedral Angle of Biphenyl and 4,4' - Disubstituted Biphenyls

The molecular conformation of biphenyl and 4,4' - disubstituted biphenyls in the absence of ligand has been studied extensively. The dihedral angle can be measured directly in the gas phase by electron diffraction and in the solid phase by x-ray crystallography. There is good agreement in the literature that the dihedral angle of biphenyl in the gas phase is 42° - 45° (48, 49) and that the biphenyl in the crystal is planar (50, 51). Many researchers have used molecular orbital theories to calculate the theoretical dihedral angle in the isolated molecule, which would best correspond to the gas phase. Such calculations have yielded minimum potential energies at dihedral angles of 35° (52), 40° (53), and 42° (54).

Results for the dihedral angle of biphenyl in solution are widely conflicting. This confict is not unexpected because the dihedral angle in solution cannot be measured directly but must be inferred from other data. A review by Zaraiskii (55) outlined the sources of conflict in the literature reports. Several analytical methods that have been applied to biphenyl in solution depend upon measurements of extent of conjugation between the rings, e.g. ultraviolet spectroscopy (56). In the case of biphenyl, however, the degree of conjugation between rings is dependent on both the dihedral angle and the length of the C-C interannular bond. Experimental data show that the bond length paradoxically decreases as the molecule goes from a planar configuration in the crystal (51, 57) to a 45° dihedral angle in the gas phase (48 b, c, d). Therefore, one cannot predict the dihedral angle from degree of conjugation data alone. Methods such as infrared (IR) and Raman spectroscopy that rely on symmetry group theory for interpretation of the experimental data are more sound methods. These methods,

however, lead only to qualitative descriptions , i. e. whether the molecule is planar or non-planar.

Another source of conflict in the literature is neglect of the effect of solvent on the dihedral angle. Wilk (58) investigated the IR spectra of biphenyl and several 4,4'-disubstituted biphenyls in the solid phase and in solution as films. Carbon disulfide and chloroform were the non-hydrogen bonding solvents used, and pyrrolidine was used as a hydrogen bonding solvent. In general, there existed some non-planarity in the non-bonding solvents. In pyrrolidine, the molecules assumed a more planar configuration than they had in the non-bonding solvents. The evidence did not show that all molecules were completely planar in the pyrrolidine.

Michelsen and coworkers (59) examined some 4,4'-disubstituted biphenyls in chloroform, dichloromethane, benzene, and heptane by IR and Raman spectroscopy. They report some non-planarity for all compounds, but do not specify which solvents were used for each compound. Pasquier and Lebas (60) observed non-planarity in biphenyl in solution when examined by IR and Raman spectroscopy.

These compounds have also been examined by proton nuclear magnetic resonance (^{1}H nmr) spectroscopy. Again, this is an indirect method of determination of dihedral angle because molecular orbital theory must applied to the experimental chemical shift data. Kurland and Wise (61) examined the ^{1}H nmr spectra of 4,4'-disubstituted biphenyls in carbon disulfide, equimolar deuterated chloroform / carbon tetrachloride mixture, methylcyclohexane, and chloroform. A potential energy curve was calculated from the experimental chemical shift data, resulting in a minimum of this curve at a dihedral angle of 90°. This minimum was very shallow, indicating much free rotation about the interannular bond. A ^{1}H nmr study in deuterated chloroform by Mayo and

Goldstein (62) determined that at 28° C, biphenyl essentially undergoes free rotation. Tarpley and Goldstein (63) observed free rotation of 4,4'-disubstituted biphenyl compounds in perdeuterated benzene at 38°C when measured by ^1H nmr.

It is clear that the literature on the dihedral angle of biphenyl is extensive and conflicting. The best consensus that can be reached is that there is some free rotation about the interannular bond at temperatures near 28°C and that the equilibrium dihedral angle is greater than 0°. The exact value of that angle cannot be determined directly by current analytical methods.

In light of the literature data, a few observations can be made concerning the experimental data presented in this work. From the literature data we see that biphenyl and disubstituted biphenyls, in solution in the absence of ligand, show some free rotation and a small nonzero equilibrium dihedral angle. The Hammett plots of a_{XX} for the disubstituted benzene and disubstituted biphenyl series show ρ values that are not significantly different. We can then conclude that the biphenyl substrates in the complex exhibit as much conjugation between sites as the benzene substrates in the complex, when the compounds are considered as a series. We are, however, unable to conclude that the biphenyl substrates in their complex forms assume a planar configuration because, as discussed earlier, the dihedral angle is dependent on both degree of conjugation and the C-C interannular bond length.

E. Summary

Equilibrium constants for 1:1 and 1:2 binding of α-cyclodextrin and a series of symmetrical 4,4'-disubstituted biphenyl compounds were determined in 0.1 N aqueous solution at 25° C. The data were analyzed according to a proposed binding site model and the interaction parameter a_{XX} was determined. A Hammett correlation of a_{XX} was made and the ρ value from that plot was compared with the ρ value for the benzene series examined by Pendergast. The ρ values for those two series were found to be not significantly different. We can therefore conclude that the extent of electron delocalization between binding sites in the biphenyl series is similar to that of the benzene series in these cyclodextrin complexes.

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VII APPENDICES

A. Computer program for the least squares analysis of eq. 20

The computer program for the least squares analysis of eq. 20 is reproduced with the solubility data entered for the biphenyl compound as an example. Within the program, [L] is calculated at each experimental L_t according to eq. 19. This program and the program in Appendix B were written on a Digital Equipment Corporation Rainbow computer in BASIC language.

```
10      Open "r", 1, "Andrea5.bas"
15      lprint "input values"
        (In lines 20 and 40, I am inputting the beginning estimate of K11 and K12)
20      input "K11 = ";K1
30      lprint "K11 = ";K1
40      input "K12 = ";K2
55      lprint "K12 = ";K2
        (In line 60, I am inputting the number of points)
60      M = 10
        (In line 70, I am inputting the mean experimental So)
70      SO = 4.0E-05
        (In line 75, I am inputting the experimental variance of So)
75      VS = 4.0E-12
80      lprint
85      lprint
90      lprint "LT";TAB(15);"ST exp";TAB(30);"ST calc";TAB(45);"L";TAB(60);
        "(ST - SO) / L"
100     read ST,LT
        (In lines 110 to 180, I am calculating [L] at each experimental Lt, according to
        eq. 19. In line 190, I am renaming [L] as X. Note that [L] is the abcissa of the
        linear equation 20. )
110     A=8*K1*K2*SO*LT
120     B=1 + (K1*SO)
130     C=B^2
140     D=A+C
150     E=SQR(D)
```

```
160     F=-B
170     G=4*K1*K2*SO
180     L=(F+E)/G
190     X=L
        (In lines 205 to 220, I am calculating a "calculated" S_t at each L_t according to
        eq. 17. These calculated S_t values will make up the calculated curve)
205     L1=K1*SO*L
210     L2=K1*K2*SO*(L^2)
220     SC=SO+L1+L2
        (In line 700, I am calculating (S_t - S_o / [L]), the ordinate of eq. 20.)
700     Y=(ST-SO)/X
705     lprint LT;TAB(15);ST;TAB(30);SC;TAB(45);X;TAB(60);Y
        ( Lines 710 to 836 are the least squares analysis of eq. 20.)
710     X1=X1+X
720     Y1=Y1+Y
730     X2=X2+X*X
740     Y2=Y2+Y*Y
760     P=P+X*Y
770     N=N+1
780     if N<M then goto 100
790     Q=P-((X1*Y1)/N)
800     S=X2-((X1*X1)/N)
802     Z=Q/S
804     W=(Y1-(Z*X1))/N
        (In lines 806 and 808, I am calculating the improved estimates of K_11 and K_12
        for a single iteration.)
806     K3=W/SO
808     K4=Z/W
812     A1=Y2-((Y1^2)/N)
814     A2=(Q^2)/S
816     A3=A1-A2
818     A4=A3/(N-2)
820     B1=A4/S
822     A6=(X1/N)^2
824     B2=A4*((1/N)+(A6/S))
826     C1=VS/(SO^2)
828     C2=B2/W^2
        (In line 830, I am calculating the standard deviation of K_11, according to
        eq. 22.)
830     SJ=(SQR(C1+C2))*K3
832     C3=B1/(Z^2)
        (In line 836, I am calculating the standard deviation of K_12, according to
        eq. 23.)
836     SK=(SQR(C2+C3))*K4
840     lprint
843     lprint
844     lprint "output values"
```

```
845   lprint "slope = ";
846   lprint "variance of slope = ";B1
850   lprint "intercept = ";W
851   lprint "variance of intercept";B2
877   lprint
878   lprint
```

(In lines 879 and 882, I am printing the improved values of K_{11} and K_{12} for a single iteration. In lines 880 and 886, I am printing their standard deviations.)

```
879   lprint "K11 = ";K3
880   lprint "std dev K11 = ";SJ
882   lprint "K12 = ";K4
886   lprint "std dev K12 = ";SK
```

(In line 890, I am calculating a_{XX})

```
890   AX=4*(K3/K4)
```

(In line 895, I am printing the improved value of a_{XX})

```
895   lprint "axx = ";AX
```

(In lines 900 to 915, I am calculating the standard deviation of a_{XX})

```
900   Q1=(SK^2)/(K4^2)
905   Q2=(SJ^2)/(K3^2)
910   Q3=SQR(Q1+Q2)
915   SA=Q3*AX
```

(In line 920, I am printing the standard deviation of a_{XX})

```
920   lprint "Saxx = ";SA
```

(In line 940, I am inputting the experimental S_t and L_t as data pairs, i.e. first S_t, L_t, next S_t, L_t, etc.)

```
940   data 4.43E-05,2.00E-03,5.00E-05,4.00E-03,5.64E-05,6.00E-03,6.51E-05,
      8.00E-03,7.03E-05,1.00E-02,9.05E-05,1.29E-02,1.74E-04,2.58E-02,3.07E-
      04,3.87E-02,4.65E-04,5.16E-02,6.76E-04,6.45E-02
950   end
```

(Note that this program will perform one iteration only. The program is rerun with the output values from lines 879 and 882 used as input values in lines 20 and 40. The program is rerun until constants K_{11} and K_{12} converge.)

B. Computer Program for First Estimates of K_{11} and K_{12}

To begin the iteration of eq. 20, first estimates of K_{11} and K_{12} are needed. The following program provides those estimates. The basis of the estimate is eq. 20, where the approximation is made that [L] = L_t . The solubility data for biphenyl is entered as an example.

```
10      open "r",1,"andrea6.bas"
50      lprint "(ST-SO)/LT";TAB(20);LT
100     Read ST,LT
        (In line 120, I am inputting the mean experimental So.)
120     SO=4.0E-05
        (In line 125 , I am inputting the number of points.)
125     M=10
        (In line 130, I am calculating the ordinate of eq. 20, but am approximating [L]
        as Lt)
130     Y=(ST-SO)/LT
        (In line 140, I am approximating the abcissa of eq. 20 , [L], as Lt)
140     X=LT
        ( Lines 150 to 250 are the least squares analysis of eq. 20.)
150     X1=X1+X
160     Y1=Y1+Y
170     X2=X2+(X^2)
180     Y2=Y2+(Y^2)
190     P=P+(X*Y)
200     N=N+1
205     lprint Y;TAB(18);X
210     if N<M then goto 100
220     Q=P-((X1*Y1)/N)
230     S=X2-((X1^2)/N)
240     Z=Q/S
250     W=(Y1-(Z*X1))/N
        (In line 260, I am calculating K11, and in line 270, K12. In lines 280 and 290, I
am      printing them.)
260     K1=W/S
270     K2=Z/(K1*SO)
275     lprint "estimate from (ST-SO)/LT vs. LT :"
280     lprint "K11 = ";K1
290     lprint "K12 = ";K2
```

292 lprint
(In line 300, I am inputting the experimental S_t and L_t as data pairs in the form of: first S_t, L_t, next S_t, L_t, etc.)

300 data 4.43E-05,2.00E-03,5.00E-05,4.00E-03,5.64E-05,6.00E-03,6.51E-05, 8.00E-03,7.03E-05,1.00E-02,9.05E-05,1.29E-02,1.74E-04,2.58E-02,3.07E-04,3.87E-02,4.65E-04,5.16E-02,6.76E-04,6.45E-02

400 en
(Note that these estimates will be used as input values in the first iteration of eq. 20. They will be input into lines 20 and 40 in the program in appendix A.)