EFFECTS OF ACYL GROUP VARIATIONS ON COMPLEXING OF CARBOXYLIC ACID DERIVATIVES WITH XANTHINES

by BERRY JAMES KLINE

(Under the supervision of Associate Professor Kenneth A. Connors)

A recent study on the inhibition of the alkaline hydrolysis rate of methyl trans-cinnamate by some heterocyclic ligands (J. A. Mollica, Jr. and K. A. Connors, J. Amer. Chem. Soc., 89, 308 (1967)) ascribed the inhibitions to a specific complexation interaction between the ester and the heterocyclic molecules, resulting in reduction of the susceptibility of the ester to hydroxide-ion catalyzed hydrolysis. This study has been extended by investigating the effects of acyl group variations on the complexing tendency of some carboxylic acid derivatives with theophylline and its anion. Theophylline, imidazole, 8-bromotheophylline, and 8-nitrotheophylline were tentatively selected as ligands, but because of multiple complexes and experimental difficulties, only the results with theophylline were considered significant in reaching conclusions. These compounds were selected as substrates: methyl cis-cinnamate, methyl benzoate, methyl crotonate, methyl acetate, trans-cinnamamide, benzamide, methyl 2,6-dichloro-trans-cinnamate, methyl p-nitro-trans-cinnamate, methyl 1-naphthoate, methyl 2-naphthoate, and methyl hydrocinnamate. The spectral, solubility, and kinetic

techniques were used to evaluate the apparent 1:1 stability constants, disagreement among them being taken as an indication of non-1:1 stoichiometry. Measurements were generally made in aqueous buffers at 25.0° and ionic strength 0.3.

Methyl cis-cinnamate and theophylline interact with a stability constant about 50% of that for the interaction between methyl trans-cinnamate and theophylline. The difference is ascribed to the loss of molecular planarity in the cis isomer. Methyl benzoate interacts with theophylline to about the same degree as does methyl trans-cinnamate. Methyl crotonate interacts with theophylline to a very small degree. This result is taken as an indication of the importance of the aromatic ring in the complexation interaction. Other results which indicate the importance of the aromatic ring are those for the interaction between theophylline and cinnamamide, benzamide, methyl 1-naphthoate and methyl 2-naphthoate. Methyl acetate did not appear to complex with theophylline. Methyl hydrocinnamate apparently interacted to a small degree with theophylline, but some unusual spectral changes were noted, and a tentative explanation for these spectral anomalies is offered. Despite its lack of planarity, methyl 2,6-dichlorocinnamate complexes with theophylline to a larger degree than does methyl trans-cinnamate. This increased interaction may be a result of dispersion forces between theophylline and the chlorine atoms. Methyl

p-nitrocinnamate interacts with theophylline to about the same degree as does methyl trans-cinnamate, but it is postulated that these substrates form qualitatively different complexes, and therefore the stability constants are not directly comparable.

Four possible structures for the complex between methyl trans-cinnamate and theophylline are proposed. Their relative merits are discussed and some of the forces which may be contributing to the stability of the complex interactions are suggested.

Appended is a study which illustrates the relative catalytic and inhibitory effects of imidazole on the hydrolysis of ethyl acetate as a function of pH. Imidazole catalyzes the hydrolysis of ethyl acetate at neutral pH while retarding the hydrolysis at high pH, presumably due to a solvent effect. At some intermediate pH the two effects may cancel each other so that no effect on the hydrolysis rate would be observed.

APPROVED Kenneth A. Connors

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TO

Darlene and Michele

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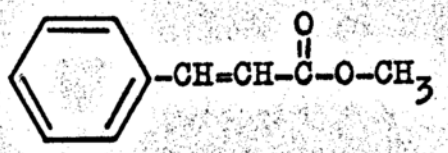
I. INTRODUCTION

A. General Background.--The rate of alkaline hydrolysis of methyl trans-cinnamate (I) is inhibited by imidazole (II) and by xanthines (III) (1). This inhibition has been attributed to a specific complexation interaction between the ester and the heterocyclic molecules, resulting in reduction of the susceptibility of the ester to hydroxide ion-catalyzed hydrolysis through steric



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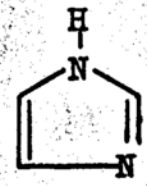

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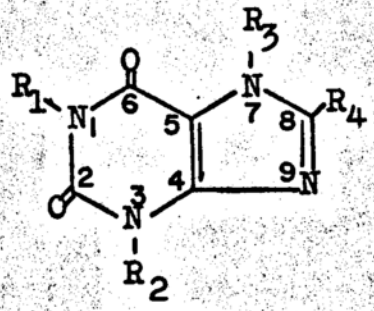

III

Theophylline: $R_1 = R_2 = CH_3$, $R_3 = R_4 = H$

Caffeine: $R_1 = R_2 = R_3 = CH_3$, $R_4 = H$

8-Chlorotheophylline: $R_1 = R_2 = CH_3$, $R_3 = H$, $R_4 = Cl$

Theophylline 7-acetic acid: $R_1 = R_2 = CH_3$,
$R_3 = CH_2\text{-}COOH$, $R_4 = H$

and/or electronic modifications (1). The phenomenon of rate modification by complex formation is well documented; see (1) and references therein. For example (and of possible practical importance to the pharmacist), the inhibition of the alkaline hydrolysis of some local anesthetics such as benzocaine by complexation with xanthines (2,3), macromolecules, and amides (4) has been demonstrated. The reasons for these inhibitions are not as readily accounted for as are the inhibitory and catalytic phenomena produced by α- and β-cyclodextrins (5). These large molecules form inclusion compounds, with the extent of inclusion of the substrate molecule into the cavities of the cyclodextrins and the degree of ionization of the hydroxyl groups on the cyclodextrins determining the direction and magnitude of the rate effects. Hart and coworkers presented evidence that the alkylation of phenols was inhibited by ethers through a complexation mechanism (6). Dioxane at a concentration of 14 mole percent reduced the apparent rate constant of phenol alkylation to about 3% of its value in the absence of dioxane. Evidence was presented supporting the existence of 2:1 and 1:1 phenol:dioxane complexes. In 1930, Allen published the results of a study in which he measured the rate of saponification of methyl acetate by sodium hydroxide in the presence of various neutral salts (7). His data indicate a 1.6% retardation by 0.1 N sodium lactate, 6.7% retardation by 0.1 N sodium glycollate, and a

33.4% retardation by 0.1 N sodium salicylate. It is not difficult to rationalize the effects by sodium lactate and glycollate as a general solvent or ionic strength effect. In view of the percent inhibitions we have observed in our systems, however, the result with sodium salicylate suggests to us a possible specific complexation interaction between the ester and the salicylate anion which reduced the susceptibility of the ester to alkaline hydrolysis.

It is interesting to note that in none of the previously discussed references is proof offered as to the forces involved in the postulated complexation interactions. This reflects the difficulty in experimentally determining and measuring such forces, which are much weaker than covalent bond forces. That a shift in the spectrum of methyl trans-cinnamate was observed in the presence of the complexing agents hinted that charge-transfer forces may be operative in these interactions, although hydrogen bonding, hydrophobic bonding, and dipole interactions could not be excluded (1). The authors also suggested that the planar ester and ligand molecules interacted with their molecular planes parallel. However, it was impossible to describe the exact geometry of the complex (or complexes) from the data available.

Many interesting questions were raised by this study with methyl trans-cinnamate and the heterocyclic ligands.

For example, do the theophylline and theophylline anion complexes with methyl trans-cinnamate have the same geometry? Why does the anion interact with a stability constant of about 12 M^{-1} while the neutral theophylline molecule gives a constant of about 24 M^{-1}? Can the structural analogy of the caffeine-methyl trans-cinnamate system, which possesses no possibilities for hydrogen bonding, be extended to theophylline and methyl trans-cinnamate, where hydrogen bonding is possible? Does 8-chlorotheophylline anion, which interacts with the ester more strongly than does theophylline, do so because of dispersion forces between the chlorine atom and the ester, or is the stronger interaction due to the electron withdrawing effects of the chlorine on the theophylline molecule to which it is attached? By what mechanism does complexation reduce the susceptibility of the ester molecule to hydroxide ion-catalyzed hydrolysis? Is the rate modification due to steric alterations, electronic modifications, or some combination of the two? We decided that the answers to many of these questions might be apparent if the structure of the complex was known.

Knowledge of the stereochemistry involved in the interaction of imidazole with methyl trans-cinnamate might also provide a key to some aspects of enzyme specificity. The imidazole moiety is known to reside in the active site of chymotrypsin as part of the amino acid, histidine. It

is possible that this imidazole molecule is involved in the enzyme-substrate complex, the stereochemistry of this interaction being such that in one case the labile group of the substrate is positioned adjacent to a catalytic function in the active site, and in another case the complex geometry could be such that the labile group of the substrate is adjacent to a catalytically inactive function. An alternate possibility for the case where a substrate appears unreactive toward an enzyme is that the substrate-imidazole complex may be unreactive toward the catalytic function just as the methyl trans-cinnamate-imidazole complex appears to be unreactive toward hydroxide-ion catalyzed hydrolysis. Thus it was decided that the purpose of this study would be to obtain information bearing on the structure of the complex formed by the interaction of methyl trans-cinnamate with some of the heterocyclic molecules selected from reference (1).

The structures of many inorganic complexes have been determined and described in terms of molecular orbital theory (8,9). However, examination of chapter three of Andrews and Keefer's treatise (10) illustrates how little is known about the structure of organic complexes, especially those which exist only in solution. X-ray diffraction studies have made a significant contribution to the determination of the structures of crystalline complexes. Andrews and Keefer, however, question the validity of the appealing assumption that the structure

of a complex is the same in the crystalline state as in solution. For example, it is generally accepted that the benzene-halogen complexes interact in a 1:1 stoichiometry in solution. However, X-ray data on crystalline benzene-halogen complexes give evidence of a coordination number greater than one in the solid state (10).

As a point of reference, it is convenient to propose a model for the methyl trans-cinnamate-heterocyclic ligand complex and then to modify it as the results of experiments dictate such changes. Since X-ray data on methyl trans-cinnamate, imidazole, and the xanthines show these molecules to be planar, a reasonable assumption is that the components of the complex interact with their molecular planes parallel. There is much evidence in the literature to support this type of interaction. Murrell has suggested that maximum overlap of donor and acceptor orbitals occurs in chloranil-alkylbenzene complexes when the two molecular planes are parallel (11). Crystallographic studies indicate that π donor-π acceptor complexes form by alternate stacking of the planes (12). The stability constant for the interaction of s-trinitrobenzene with biphenyl derivatives decreases with ortho substitution on one of the biphenyl rings (13). This has been attributed to the non-planarity of the double ring system produced by ortho substitutions, thus inhibiting the preferred parallel orientation of the interacting molecules. Nakano and Higuchi have shown that benzamide and theophylline interact with a stability

constant of 12 M^{-1} while N,N-dimethylbenzamide and theophylline give a stability constant of 2 M^{-1} (14). These workers postulate that benzamide can interact with the planar theophylline molecule more readily than does the N,N-dimethyl derivative because benzamide is planar while the disubstituted amide is not. With this model in mind, let us examine the experimental approach planned to obtain information concerning the geometry of the complex.

B. Plan of Research.--One of the most powerful approaches available to the chemist seeking mechanistic insight is the one utilizing structural variations in reactants, with observation of the effects these structural changes lead to in the experimental quantities, such as equilibrium or rate constants. This classical approach has been applied to the complex formation between xanthine ligands (mainly theophylline) and methyl esters; the structural variation was thus concerned with the acyl portion of the carboxylic acid derivative: R in $R\text{-}COOCH_3$. The reference ester was, of course, methyl trans-cinnamate. It was hoped that this would show which features of the trans-cinnamoyl group control the stability of the complex. Through the proper design of experiments, it should be possible to find some bond angles, lengths, and configurations which are critical to the interaction of the ester with these ligands.

Of primary importance was the selection of a ligand (or ligands). It was desirable that the ligand interact with an apparent 1:1 stoichiometry, not forming multiple complexes. It was also desirable that the cinnamate complex exhibit a relatively large stability constant, so that changes in this constant might be measured relatively accurately. Of the ligands that have been studied (1), theophylline, theophylline 7-acetic acid, caffeine, and 8-chlorotheophylline interacted most strongly with methyl trans-cinnamate. However, caffeine was eliminated because of the complications introduced by self-association of this ligand (15). Theophylline 7-acetic acid was suspected of producing multiple complexes (1). The lability of the chloride in 8-chlorotheophylline was questioned when reproducibility of some of the data proved difficult to achieve. For these reasons theophylline was the ligand of choice, since it met most of the criteria. It was both an advantage and a disadvantage that theophylline could be studied in the ionized and the unionized form. Since other investigators in this laboratory had obtained preliminary data indicating that 8-nitro- and 8-bromotheophylline anions gave larger stability constants than theophylline, these two ligands were also selected for tentative study. When possible, studies with imidazole were done since they were not as difficult experimentally as were studies with the xanthines.

The following substrates were selected for study, all of them representing some alteration in the basic acyl structure of the methyl trans-cinnamate molecule: methyl cis-cinnamate, methyl benzoate, methyl acetate, methyl 2,6-dichlorocinnamate, methyl p-nitrocinnamate, methyl 1-naphthoate, methyl 2-naphthoate, methyl hydrocinnamate, and styrene. Cinnamamide and benzamide were added later when a complexing study involving these compounds and theophylline appeared in the literature (14); the results of this study aroused our interest because the stability constants reported seemed inconsistent with the results we had obtained for benzoate and cinnamate esters. The kinetic, solubility, and spectral methods of studying the interactions were utilized, disagreement among them being taken as an indication of non 1:1 stoichiometry.

II. EXPERIMENTAL

A. Materials.--Methyl hydrocinnamate was prepared by treating hydrocinnamoyl chloride (Eastman Organic Chemicals) with methanol. The product was purified by distillation under reduced pressure, bp 73.5° (3 mm); bp 233° (lit.(16) 232°). The purity was checked by determination of the saponification equivalent. Found: 163.7, 164.2. Theoretical: 164.21.

Hydrocinnamic acid (Eastman Organic Chemicals) was recrystallized from water, mp 47-48° (lit.(17) 47.5-48°).

Methyl benzoate (Matheson, Coleman and Bell) was distilled through a packed column, bp 196.5-197°, n_D^{21} 1.514 (lit. (16), bp 199°, n_D^{20} 1.517).

Methyl acetate was prepared by treating acetic anhydride with methanol. Final purification was accomplished by distilling the crude ester from phosphorous pentoxide through a packed column, bp 56.5° (lit. (18), 57.1°).

Methyl crotonate (Matheson, Coleman and Bell) was purified by distillation through a packed column, bp 117-118° (744 mm) (lit. (16), 119°).

The synthesis of cis-cinnamic acid was attempted by the following three methods: (i) a series of stereospecific reactions which convert phenylpropiolic

acid into cis-cinnamic acid (19); (ii) ultraviolet irradiation of dilute solutions of trans-cinnamic acid (20-22); (iii) catalytic hydrogenation of phenylpropiolic acid on Lindlar-type catalysts (23-28). Such poor yields were obtained by method (i) that it was abandoned. Although the literature abounds with reports on the production of ring-substituted cis-cinnamic acids by ultraviolet irradiation of the corresponding trans acids, we failed in all attempts to convert trans-cinnamic acid to the cis compound by this method. The only method to meet with moderate success was that of catalytic hydrogenation of phenylpropiolic acid. A large number of hydrogenation experiments was carried out before reasonable yields were obtained. The previously cited references on this method did not list all of the critical experimental details such as temperature, solvent, rate of hydrogen up-take, substrate/catalyst ratio, and catalyst poisoning agent, if used. Better yields were obtained using the Parr low-pressure apparatus than with the atmospheric hydrogenation equipment. Details of this procedure are as follows.

A solution of sodium phenylpropiolate was prepared by dissolving 10 gm of phenylpropiolic acid in 90 ml of water containing 4.1 ml of saturated aqueous sodium hydroxide solution. This was placed in a 250 ml Parr hydrogenation bottle with 0.300 gm of Lindlar's

catalyst* and five drops of synthetic quinoline (J. T. Baker Chemical Co.). The hydrogenation was initiated by shaking at an initial pressure of 40 psi at room temperature. In this particular experiment, the pressure dropped to 34 psi over a period of 1.5 hours and was constant for 30 minutes. The calculated pressure drop for this system was 5.8 psi.

The hydrogenation mixture was removed from the Parr bottle and the catalyst filtered off. The filtrate was acidified with concentrated hydrochloric acid to a pH of 1 or less (pH paper). The precipitated free acid was extracted with three portions of ether, the combined ether extracts dried with anhydrous magnesium sulfate, and the ether evaporated to give a yellow, low-melting solid, which was purified by a modification of Liebermann's aniline salt precipitation (30). This method utilizes the fact that the aniline salt of trans-cinnamic acid is soluble in cold benzene while that of cis-cinnamic acid is insoluble. Approximately 10 parts of product was dissolved in 15 parts of cold benzene and 7 parts of aniline was added (quantities are not critical). The precipitate which formed was suction-filtered, and more aniline was added to the filtrate to obtain a second crop of crystals. The two crops were combined and the

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*Lindlar's catalyst was prepared according to Reference 29, which contained a translated portion of Lindlar's original monograph (27).

cis-cinnamic acid was released from this solid with aqueous 5% hydrochloric acid. The free solid acid was extracted with three portions of ether, the combined ethereal extract dried ($MgSO_4$), and the ether evaporated. The residue was recrystallized twice from ligroin to yield 6.2 gm of cis-cinnamic acid, mp 67-68° (lit. (31), 68°). Cis-cinnamic acid exists in at least three polymorphic forms with melting points of 68°, 58°, and 42° (32). Although we never obtained the 42° polymorph, occasionally the 58° form would crystallize, but the reason for this was never clear.

Methyl cis-cinnamate was prepared by treating cis-cinnamic acid with diazomethane in ether (33). The ester was purified by distillation under reduced pressure, bp 126.5-128° (17-18 mm) (lit. (34), 129-130° (corr.)/17 mm; (28) 125-130°/0.01 mm); bp 247°; mp -3° to -2° (lit. (19), -3.5°; (28) -2°). The nuclear magnetic resonance spectrum in deuterated chloroform gave a singlet at τ 6.35 (methyl protons), two pairs of doublets at 4.10 and 3.12 (olefinic protons, J = 13 Hz), and a multiplet at 2.61 (aromatic protons). Integration gave a 3:1:1:5 ratio for the four types of protons in the molecule.

Methyl 2-naphthoate was prepared by treating 2-naphthoyl chloride (Eastman Organic Chemicals) with methanol in pyridine. The ester was purified by recrystallizing twice from ethanol-water, mp 78° (lit. (35), 77°).

Methyl 1-naphthoate was prepared by treating 1-naphthoyl chloride (Eastman Organic Chemicals) with methanol in pyridine. The ester was purified by distillation under reduced pressure, bp 171-172° (17 mm) (lit. (36), 159-160° (10 mm)).

Trans-cinnamamide was prepared by treating cinnamoyl chloride with ammonium hydroxide. The amide was purified by four recrystallizations from ethanol-water using Norit during the first recrystallization, mp 147° (lit. (35), 147°, 144°, 141°).

Benzamide had been prepared by K. A. Connors, mp 130° (lit. (35), 130°).

Styrene (Aldrich Chemical Co.), to which 0.5% hydroquinone had been added, was distilled through a packed column under reduced pressure, bp 66-67° (49-51 mm) (lit. (37), 65° (50 mm)). It was stored under nitrogen in a dark bottle at refrigerator temperatures.

Methyl 2,6-dichloro-trans-cinnamate was prepared by refluxing 2,6-dichloro-trans-cinnamic acid (Aldrich Chemical Co.) with methanol for ten hours in the presence of a small amount of sulfuric acid. An attempt was made to purify the ester by vacuum sublimation. During this attempt, however, the crude ester melted since it was necessary to warm it to 110° before any crystals appeared to collect on the dry ice-acetone cold finger at a pressure of one millimeter. This could be described as a short-path vacuum distillation although at no time did the melted

ester appear to boil. The compound collected on the cold finger as white needles, mp 51.5-52°.

Anal. Calcd. for $C_{10}H_8Cl_2O_2$: C, 51.98; H, 3.49; Cl, 30.69. Found: C, 52.11; H, 3.55; Cl, 30.72.

The nuclear magnetic resonance spectrum in deuterated chloroform yielded a singlet at τ 6.15 (methyl protons), two sets of doublets at 3.42 and 2.20 (olefinic protons, J = 16 Hz) and a multiplet at 2.72 (aromatic protons). The integration yielded a 3:1:1:3 ratio for the four types of protons in the compound.

The acid chloride of p-nitro-trans-cinnamic acid (Eastman Organic Chemicals) was prepared by refluxing the acid with thionyl chloride. Methyl p-nitro-trans-cinnamate was then prepared by refluxing the acid chloride with methanol. The solid ester was recrystallized twice from ethanol, mp 163° (lit. (35), 161°).

Theophylline (Merck & Co., U.S.P.) was recrystallized once from water. It was then dried at 150° for 12 hours, mp 271-272°. This was the anhydrous form as determined by non-aqueous titration in N,N-dimethylformamide with standard lithium methoxide in benzene-methanol, using thymol blue as the indicator.

Imidazole was purified as previously described (1).

8-Bromotheophylline (Aldrich Chemical Co.) was used as purchased. Its purity was found to be satisfactory by the same titration technique previously described for

theophylline.*

8-Nitrotheophylline dihydrate (K & K Laboratories) was used as purchased, mp 282-283°d (lit. (38), 282-283°). Potentiometric titration of the compound with standard sodium hydroxide, and loss of weight on drying at 150°, showed that (a) the compound was the dihydrate although not so labelled by the manufacturer, and (b) the compound was at least 98% pure as purchased.

Acetonitrile and isooctane were purified as described by Mollica and Connors (1). Buffer chemicals were of reagent grade quality; all water was redistilled from alkaline permanganate. Standard buffers were prepared as directed by Bates (39), while other buffers were prepared as indicated by Bates and Bower (40).

Table I lists useful spectrophotometric data for some of the materials described in this section.

B. Apparatus.--Constant temperatures were achieved with water baths controlled to a claimed precision of ±0.01° by Sargent Thermonitor Relays. Some solubility determinations were made in a bath controlled to ±0.05° by a mercury column regulator. Bath temperatures were checked with thermometers carrying A.S.T.M. or National Bureau of Standards calibration certificates.

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*This determination was carried out by Mr. Paul A. Kramer. of this laboratory.

TABLE I

Ultraviolet Spectrophotometric Data

Compound	λ_{max}	$10^3 \epsilon_{max}$	Solvent
Methyl hydrocinnamate	258 mμ	0.241	water
	259	0.196	isooctane
Methyl benzoate	231	11.4	water
	227	12.1	isooctane
Methyl cis-cinnamate	272	10.4	95% ethanol
	268	8.63	water
	272	12.1	isooctane
cis-Cinnamic acid	262	10.2[a]	95% ethanol
cis-Cinnamate ion	254	11.7[b]	0.1 N NaOH
Methyl 2-naphthoate	279	6.82	isooctane
Methyl 1-naphthoate	297	6.45	isooctane
trans-Cinnamamide	275	20.0	chloroform
	276	21.7	water
Benzamide	228	--	water
Styrene	247.5	12.4	water
	248	15.0	isooctane
Methyl 2,6-dichloro-trans-cinnamate	263	11.8	isooctane
Methyl p-nitro-trans-cinnamate	302	--	water
Theophylline	272	10.6	water

[a] Lit. (43) ϵ_{256-7} 1.288×10^4

[b] Lit. (43) ϵ_{254} 1.202×10^4

pH Measurements were made on a Sargent model DR pH meter equipped with a combination glass-calomel electrode, or on a Radiometer model 25 pH meter with scale expander and a wide range glass electrode. For all measurements the meters were standardized between pH 7.413 and 9.180 using the standard buffers previously described.

All spectrophotometric measurements were made on a Cary Recording Spectrophotometer, model 14, equipped with a thermostated cell compartment and circulating water bath that maintained temperature to $\pm 0.1°$.

Solubility determinations were carried out in an apparatus described by Mollica and Connors (1). Melting points were determined on a Fisher-Johns or Thomas-Hoover Capillary Melting Point Apparatus. For the accurate delivery of very small volumes in spectral and pH-stat experiments, a 2.0 ml micrometer burette (Roger Gilmont Instruments) was used.

C. Kinetic Measurements.--Unless otherwise noted, kinetic studies were done at 25.0° C and ionic strength 0.3, with hydroxide-ion activity established by pH measurement as described. The reaction media were aqueous phosphate or hydroxide buffers at pH's from 11 to 12.6.

Rate studies done in the absence of acetonitrile were conducted in one of two ways. If the ester was soluble enough in water, a stock solution of the ester in water was prepared to be 100 times more concentrated than the

desired initial concentration. Then this solution was diluted to 1/100 of its original concentration with a buffer of appropriate pH to initiate the reaction. For example, methyl benzoate was studied in this manner. For less soluble esters, a solution of the ester in water at the desired initial concentration was prepared. Initiation of the reaction was accomplished by adding, in the amount of one percent of the final volume, a concentrated stock solution of alkali hydroxide or buffer salts. Methyl cis-cinnamate was studied this way.

Kinetic measurements on methyl hydrocinnamate were made utilizing the second technique, but, because of the very small absorbance changes which occurred upon alkaline hydrolysis of this ester, it was necessary to use the expanded scale slide-wire (0.0-0.2 absorbance unit) on the Cary 14. Since the actual absorbance throughout the reaction was greater than 0.2, a neutral density screen (A = 0.473) was used in the reference compartment to bring the pen on scale. The pen was balanced at approximately A = 0.01 with the neutral density screen in the reference compartment and a one centimeter cell containing the ester solution in the sample compartment. The reaction was then initiated and the increase in absorbance at 260 mμ was followed.

The details of the spectrophotometric procedures used for kinetic measurements of reactions with various half-lives have been described (1) and were generally followed

during this study. The direct spectrophotometric methods were useful in studies with imidazole, since its absorption was rarely appreciable at the analytical wavelengths.

All kinetic studies employing theophylline as a ligand, and that were followed spectrophotometrically, required a preliminary isooctane extraction of the ester from an aliquot of reaction mixture taken at known time. The theophylline was not removed from the aqueous buffer by one isooctane extraction, while the ester was essentially completely extracted. The pH-stat equipment and methods have been described elsewhere (41). Rate constants were determined from plots of log $(A_t - A_\infty)$ or log $(A_\infty - A_t)$ vs. time as well as by the method of Guggenheim (42). Table II is a compilation of the second-order alkaline hydrolysis rate constants for various esters.

D. Solubility Measurements.--An amount of substrate known to be in excess of its solubility was added to each of several 4-dram screw-cap vials. To each vial was added 10.0 ml of buffer solution containing varying amounts of ligand. Parafilm® was used in addition to the screw-cap to seal the vials. The vials were tumbled in a constant-temperature water bath for 24 hours or longer.

The method of separating the excess substrate from the aqueous solution varied with the physical form of the substrate. If the substrate was a solid, the aqueous

TABLE II

Second-Order Rate Constants for Alkaline Hydrolysis

Ester	Method[a]	Initial concentration 10^3(M)	$10^2 k_{OH}$($M^{-1}sec^{-1}$)[b] Mean	N.D.[c]	n.[d]
Methyl benzoate	S	0.10	10.24	0.04	5
		0.15	8.95[e]	0.35	11
Methyl acetate	pH	23.5	26.4	2.0	10
Methyl hydrocinnamate	D	3.0	17.9	0.64	8
Methyl crotonate	pH	26.0	3.44	0.12	5
Methyl *cis*-cinnamate	S	0.16	6.55	0.12	8
Methyl *p*-nitro-*trans*-cinnamate	S	0.02	18.5[f]	--	3
			22.7[g]	--	

[a] See text for details. S - Direct spectrophotometry; pH - pH-stat; D - Differential spectrophotometry.

[b] 25.0°; ionic strength, 0.3; solvent, water; based on activity measurements (except as noted).

[c] Standard deviation.

[d] Number of determinations.

[e] Ionic strength, 0.1; solvent, water with 1% acetonitrile.

[f] 25±0.5°; ionic strength, 0.06; solvent, water with 1% acetonitrile, based on concentration measurements.

[g] Based on activity calculated from $\gamma_{OH^-} = 0.815$ for 0.05 M sodium hydroxide.

phase was removed via a pipette with glass wool covering the tip as a filter. If the substrate was a liquid which tended to pass through the glass wool during attempted separation, the following technique was adopted. A typical suction filtration apparatus was used in the form of a 500-ml suction flask, a 15- or 30-ml ultrafine sintered glass funnel, and a six inch test tube inside the flask as a receiver. The system was connected to a water aspirator, and the saturated solutions with excess substrate were filtered in the usual fashion. The liquid substrate was retained by the fritted disc of the funnel while the aqueous solution passed through readily. This method was also successful in breaking emulsions when occasionally they formed between the liquid substrate and the aqueous phase.

Analysis of solubility samples was done by one of three methods depending on the properties of the substrate and ligand. Where possible an aliquot of the aqueous phase was diluted with the appropriate amount of water or 50-50 methanol-water mixture and the absorbance measured at a wavelength where the ligand did not absorb. When the ligand absorbed light at wavelengths of analytical utility, the substrate was extracted with isooctane and the isooctane phase was then measured spectrophotometrically or, when necessary, diluted and then measured. A third method was adopted specifically for cinnamamide in the

presence of theophylline.* A 1.0 ml aliquot of the aqueous phase was made alkaline with one drop of saturated aqueous sodium hydroxide solution. This was extracted with 10.0 ml of chloroform. Then 1.0 ml of the chloroform phase was diluted to 25.0 ml with chloroform and the absorbance due to cinnamamide measured at 275 mμ.

In all solubility studies analyzed by preliminary extraction of the substrate, a portion of the vials containing no ligand was scanned spectrophotometrically before being extracted. This proved to be a check on the accuracy of the extraction technique as well as a useful means for disclosing spectral anomalies masked by the organic solvent after extraction.

Because of the relatively large initial solubility of methyl crotonate and benzamide in aqueous solution, solubility-complexation studies were conducted with these compounds as ligands and theophylline as the substrate.

Table III is a compilation of the equilibrium solubilities of the various substrates under our experimental conditions.

E. Spectral Studies.--The techniques used for spectral studies have been described (1), but some minor improvements will be discussed. The micrometer burette (as

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*This method was developed by Mr. Jordan L. Cohen.of this laboratory.

TABLE III

Equilibrium Solubilities of Substrates in Aqueous Buffer

Substrate	Analytical method[a]	Solubility x 10^3(M)[b]
Methyl cis-cinnamate	Iso-Ex	5.18 ± 0.25 5.40 ± 0.09[c] 5.05 ± 0.09[d] 4.78-5.08[e]
Methyl 2-naphthoate	Iso-Ex	0.0809-0.0835
Methyl 1-naphthoate	Iso-Ex	0.320-0.340
trans-Cinnamamide	$CHCl_3$-Ex	8.49 ± 0.12
Theophylline	Diln.	0.324-0.328
Methyl 2,6-dichloro-trans-cinnamate	Iso-Ex	0.0675-0.0742
Methyl p-nitro-trans-cinnamate	Diln.	0.0481 ± 0.0028

[a] See text for details. Iso-Ex - Isooctane extraction; Diln. - Direct dilution of aqueous phase; $CHCl_3$-Ex - Chloroform extraction.

[b] 25.0°; solvent, pH 6.4, 0.05 M aqueous phosphate buffer; ionic strength, 0.3; expressed as mean ± 1 standard deviations or as experimental range.

[c] 35.0°.

[d] 15.0°.

[e] 2.0°.

previously described) used for the delivery of small volumes of substrate-acetonitrile solutions was found to be superior in reproducibility to any pipet used.

Where the absorbance changes due to complexation were too small to be measured accurately in 1.0 centimeter cells, cells of 2.0, 5.0, and 10.0 centimeter pathlength were resorted to. At times it was necessary to increase the substrate concentrations to the point where the substrate was initially insoluble in the aqueous buffer. Upon standing for 12-24 hours, the substrate eventually dissolved as long as the final concentration did not exceed the equilibrium solubility. Where undesirable complicating reactions were possible during this dissolution interval (hydrolysis, oxidation, and isomerization), the flasks were stored at refrigerator temperatures. The samples were then brought to 25° immediately before being read spectrophotometrically.

F. Treatment of Data.--The mathematical methods, assumptions and approximations for the evaluation of stability constants by the three experimental methods have been discussed in detail elsewhere (44). They were applied in all cases to the data from this study. A summary of the final equations, plotting forms, and method of computing K_{11}', from the plots is presented here. All concentrations are molar; all methods assume a 1:1 stoichiometry.

a) Solubility Method.

$$S_t = \frac{K_{11} S_o L_t}{1 + K_{11} S_o} + S_o$$

where,

S_o = solubility of substrate in absence of ligand

S_t = total solubility of substrate in presence of ligand

L_t = total molar ligand concentration

K_{11}' = apparent 1:1 stability constant.

Plot S_t vs. L_t; then,

$$K_{11}' = \frac{\text{slope}}{\text{intercept (1-slope)}}$$

b) Spectral Method.

$$b/\Delta A = 1/K_{11} S_t \Delta a(L) + 1/S_t \Delta a$$

where,

b = cell path length

ΔA = difference in absorbances of substrate in absence and presence of (L) moles/L of ligand.

S_t = total substrate concentration

Δa = difference in molar absorptivity between the complex and the substrate and ligand; $\Delta a = a_{11} - a_S - a_L$.

(L) = concentration of uncomplexed ligand.

Plot $b/\Delta A$ vs. $1/L_t$ (where the assumption is made that $(L) = L_t$);

then,

$$K_{11}' = \text{Y-intercept/slope.}$$

c) Kinetic Method.

$$\frac{k_s}{k_s - k_s'} = \frac{1}{q_{11}K_{11}(L)} + \frac{1}{q_{11}}$$

where,

k_s = rate constant for reaction of substrate in absence of ligand

k_s' = rate constant for reaction of substrate in presence of ligand

q_{11} = fractional decrease in reactivity of complexed substrate

(L) = concentration of uncomplexed ligand.

Plot $k_s/(k_s - k_s')$ vs. $1/L_t$;

then,

$$K_{11}' = \frac{\text{Y-intercept}}{\text{slope}} = -\text{X-intercept.}$$

Where a significant amount of scatter was evident on a linear solubility plot, the method of least squares was applied to determine the best line through the experimental points. The method of weighted least squares was applied to all double reciprocal (kinetic and spectral) plots with the aid of a digital computer.* The slope and Y-intercept of the weighted least squares line are computed by the

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*The program for this numerical analysis was written by J. L. Cohen and P. A. Kramer.

following equations.

$$\text{Y-intercept} = \frac{\Sigma wx^2 \Sigma wy - \Sigma wx \Sigma wxy}{\Sigma w \Sigma wx^2 - (\Sigma wx)^2}$$

$$\text{Slope} = \frac{\Sigma w \Sigma wxy - \Sigma wx \Sigma wy}{\Sigma w \Sigma wx^2 - (\Sigma wx)^2}$$

The computation of the weighting factor, w, is based on experience with each experimental system. Our experience with these systems indicated that as the value of the quantity plotted on the Y-axis increased, the accuracy of its determination decreased. Although it is preferable to base w on the variance of the dependent variable, this information was not available from these experiments. However, it was observed that the square of the dependent variable was proportional to its variance. Thus, the value of w was computed by the formula

$$w = \frac{d^2}{\Sigma d^2}$$

where d is the denominator of the fraction plotted on the Y-axis, this denominator being the dependent variable.

III. RESULTS

A. Methyl cis-Cinnamate.--A comparison of complexing tendencies between methyl trans-cinnamate and methyl cis-cinnamate is certainly desirable. The cis compound represents a change in configuration about the double bond resulting in a loss of planarity. (The trans isomer is planar.) It would be interesting to observe the changes in complexing tendency brought about such stereochemical alterations in the basic cinnamate structure.

Methyl cis-cinnamate exhibited second-order kinetics under conditions of alkaline hydrolysis. Isomerization of the cis isomer to the trans was investigated since the cis compound is thermodynamically the less stable of the two, and equilibrium between the cis and trans acids has been shown to exist in aqueous acid solutions (25). Isomerization appears to be catalyzed by normal laboratory illumination. When flasks and vials containing solutions of the cis ester were wrapped with aluminum foil, the isomerization rate was reduced to a negligible level. In unprotected glassware, the isomerization reaction was never significant during the period of a kinetic study, the longest of which was about 3.5 hours in duration. The isomerization reaction did interfere in solubility studies where equilibration times of 24 hours and longer were necessary. Again, however, protection from light

with aluminum foil rendered the isomerization rate negligible.

The rates of alkaline hydrolysis of methyl cis-cinnamate in the presence of acetonitrile and imidazole were determined under pseudo-first order conditions directly in the spectrophotometer cell at 285 mμ. Table IV is a compilation of the relative rates in the presence of these two additives. At 0.50 M concentrations, imidazole caused a 24-25% decrease in the rate relative to the no-additive rate, while acetonitrile reduced the rate by 17-18%. This suggested to us that the effects of imidazole on the alkaline hydrolysis rate might be more than just solvent effects. However, when the data for imidazole were plotted on a $k_s/\Delta k_s$ vs. $1/L_t$ plot, a straight line intercepting the Y-axis very near the origin was obtained. The K_{11}' calculated from this plot was 0.07 M^{-1}.

Theophylline anion (hereafter referred to as theophyllinate) was observed to inhibit the rate of alkaline hydrolysis of methyl cis-cinnamate, so a kinetic-complexation study was conducted on this substrate-ligand pair. Figure 1 is an example of the first order plots obtained for this system and Figure 2 is the kinetic reciprocal plot of the apparent second-order rate constants compiled in Table V. The K_{11}' calculated from this line is 7 M^{-1}. The experimental uncertainty is about 30%. From the intercept of this plot we can compute the

TABLE IV

Relative Rates of Methyl cis-Cinnamate Alkaline Hydrolysis in the Presence of Various Imidazole and Acetonitrile Concentrations

Imidazole (M)	k_s'/k_s[a]
0.10	0.95
0.15	0.93
0.20	0.92
0.25	0.88
0.35	0.83
0.50	0.75
0.50	0.76

Acetonitrile (M)	
0.10	0.99
0.10	1.00
0.35	0.86
0.35	0.90
0.50	0.83
0.50	0.82

[a]Second-order rate constants based on hydroxide ion activity; pH 11.6 phosphate buffer, 25.0°, $\mu = 0.3$, $k_s = 6.55 \times 10^{-2} M^{-1} sec^{-1}$, ester concentration = 1.6×10^{-4} M.

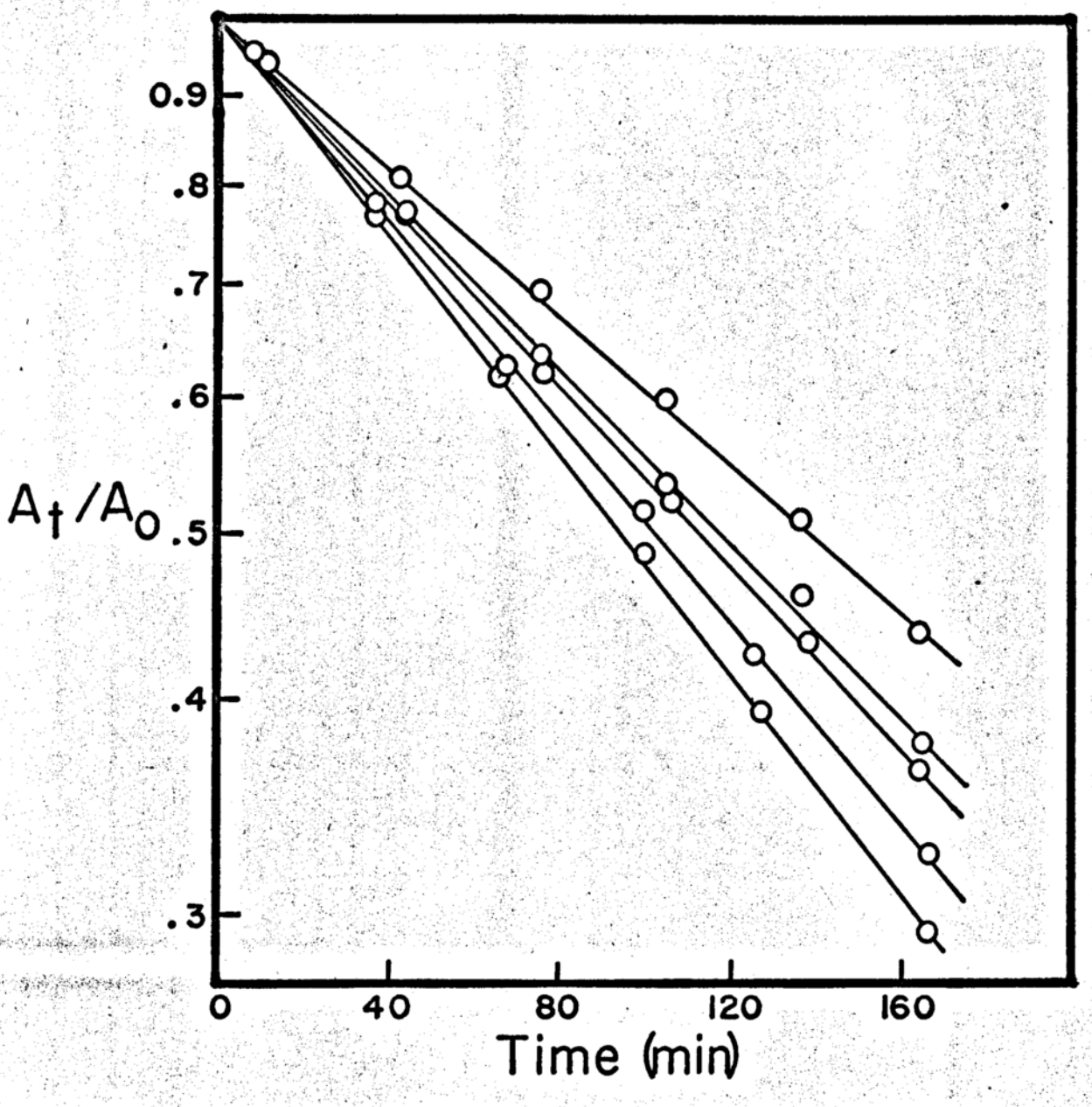


Figure 1. First-order plots for the alkaline hydrolysis of methyl cis-cinnamate in the presence of theophyllinate. The lines from top to bottom are for the following molar theophyllinate concentrations: 0.075, 0.050, 0.035, 0.020, 0.000.

TABLE V

Apparent Second-Order Rate Constants for the Alkaline Hydrolysis of Methyl cis-Cinnamate in the Presence of Theophyllinate

Theophyllinate ($M \times 10^2$)	$10^2 k_s'$ ($M^{-1} sec^{-1}$)[a]
0.0	6.29, 6.14
2.0	5.67, 5.51
2.5	5.49, 5.02
3.5	5.10
5.0	4.93
7.5	4.05

[a] pH = 11.3, μ = 0.3, 1% CH_3CN, 25.0°, based on hydroxide-ion activity, ester concentration = 2.7×10^{-4} M.

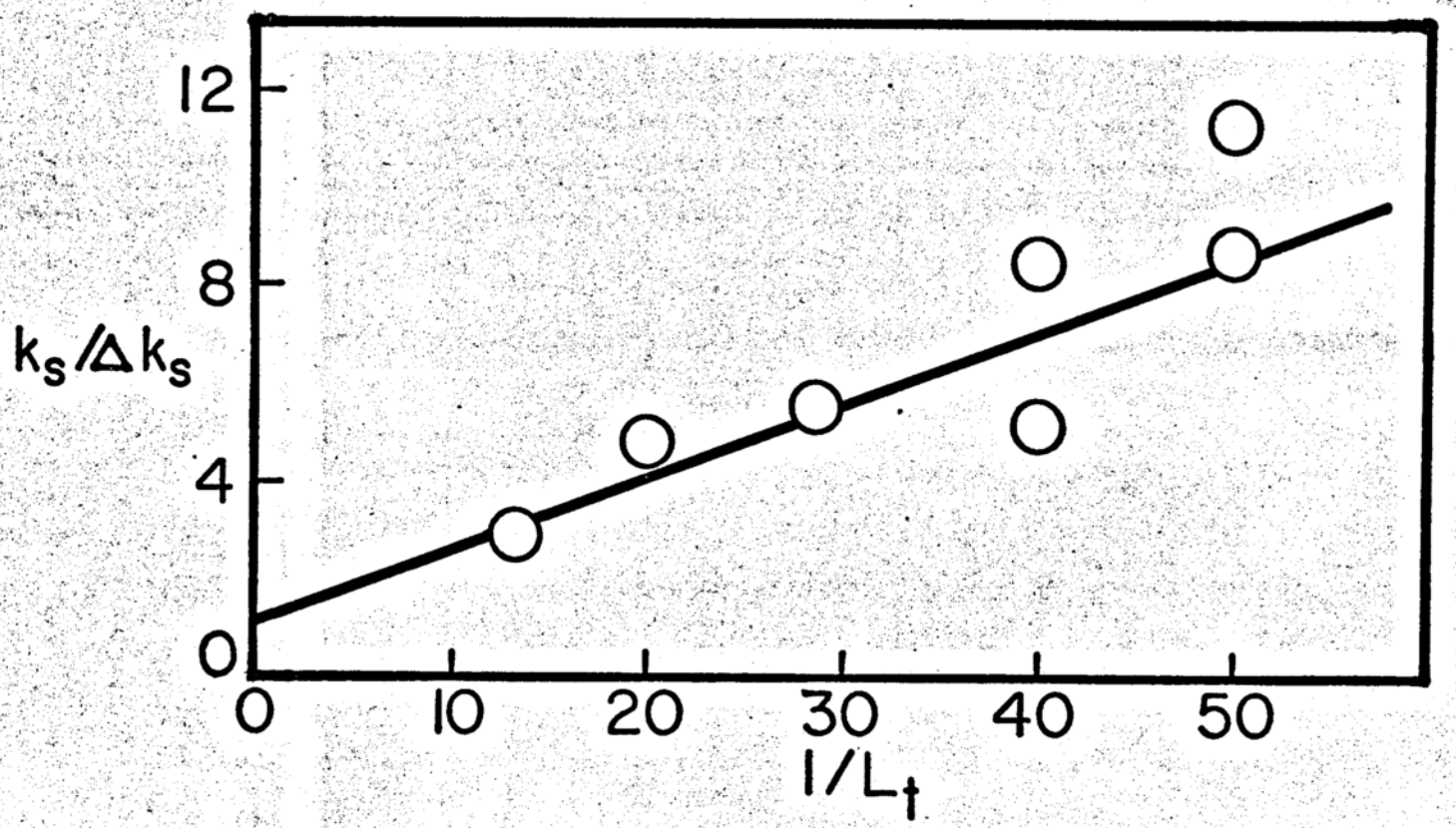


Figure 2. Plot of kinetic data for the methyl cis-cinnamate-theophyllinate system (see Table V for conditions).

value of q_{11}. This symbol represents the fractional decrease in reactivity of the complexed substrate. Mathematically it is defined:

$$q_{11} = 1 - k_{11}'/k_s$$

where, k_{11} is the rate constant for reaction of the complex with the reagent (in these systems, hydroxide ion), and k_s is the rate constant for reaction of the substrate with the reagent. Within the limits of experimental error the q_{11} of 0.96 (1/Y-intercept) obtained for the methyl cis-cinnamate-theophyllinate system indicates that the complex has very little susceptibility, if any, to hydroxide-ion catalyzed hydrolysis.

The interaction of methyl cis-cinnamate with theophylline was studied by the solubility and spectral techniques. The results of the solubility study are given in Table VI and Figure 3. The stability constant computed from the least-squares line through the points is 11 M^{-1}. Even though the experimental uncertainty in this system is about 10%, we cannot ignore the possibility that the data in Figure 3 could perhaps be better fitted by a curve with a negative trend at high theophyllinate concentrations. The reason for this type of curvature in solubility plots is not always clear (45). Regardless of the reason, a sizable increase in the solubility of methyl cis-cinnamate is obvious, this being about 40% at 0.035 M theophylline.

TABLE VI

Solubility of Methyl cis-Cinnamate in the Presence of Varying Amounts of Theophylline[a]

Theophylline ($M \times 10^2$)	Me cis-Cinnamate ($M \times 10^3$)
0.000	5.18[b]
0.350	5.33
1.05	5.71
1.40	5.80, 5.88
1.75	6.12, 6.09
2.10	6.29, 6.30
2.45	6.31, 6.46
2.80	6.65
3.15	6.68, 6.73
3.50	6.76, 6.89

[a] 25.0°; pH 6.4 phosphate buffer, $\mu = 0.3$.

[b] Mean from Table III.

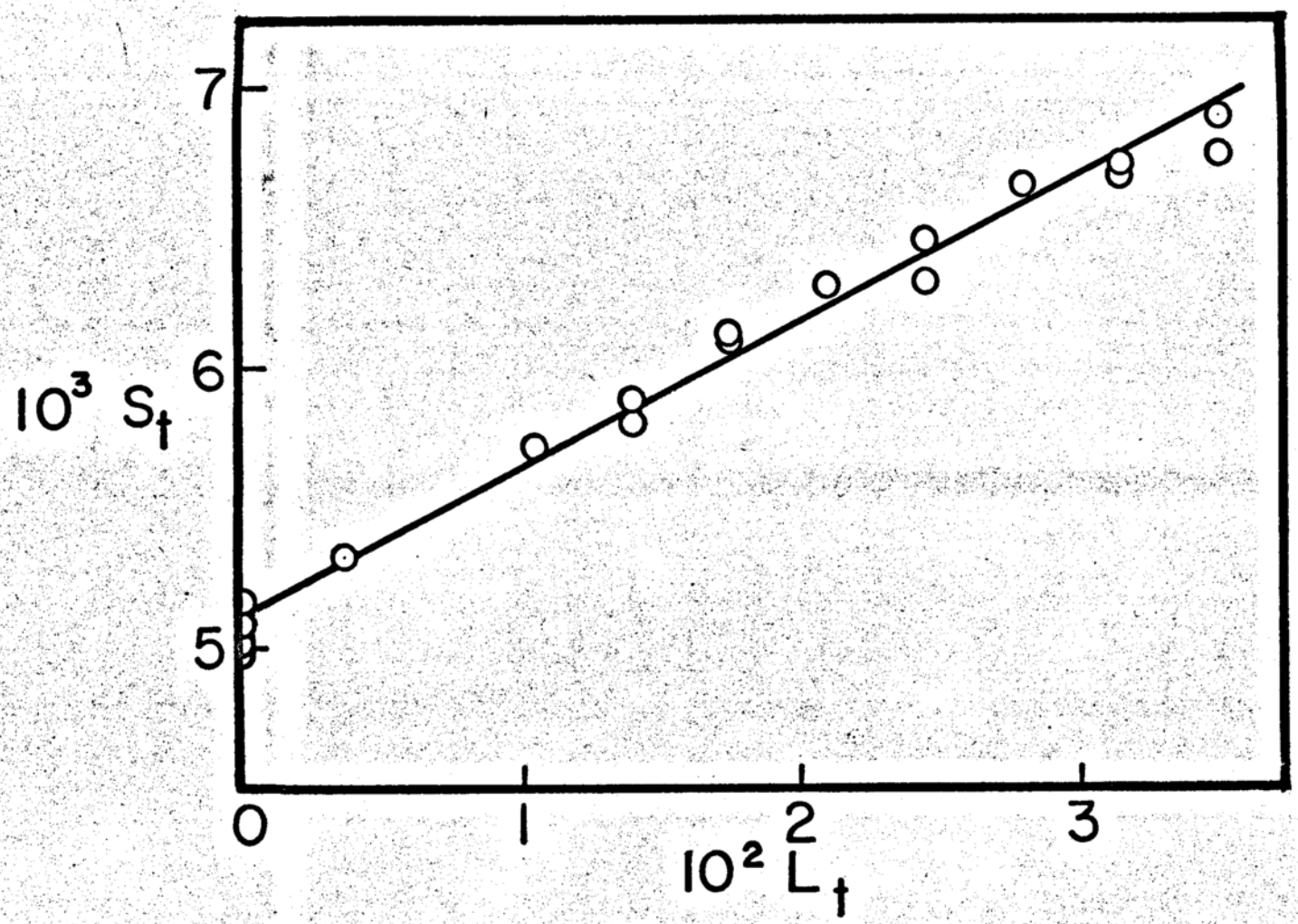


Figure 3. The apparent solubility of methyl cis-cinnamate as a function of theophylline concentration at 25.0°. (Data are given in Table VI).

The curvature, if real, will not change the conclusions which we will later make from this result.

In an effort to determine some of the thermodynamic parameters controlling the interaction of methyl cis-cinnamate with theophylline, solubility studies were also conducted at 2°, 15°, and 35°, the results being shown in Table VII. The stability constants obtained from these studies were then plotted on a van't Hoff' type of plot as shown in Figure 4. For purposes of comparison, the results of similar studies with methyl trans-cinnamate and theophylline are included on this plot.*

It is obvious that the data for the cis compound do not fit the van't Hoff relationship nearly as well as do the data for the trans ester. Except for the study at 2°, the investigations were done at least in duplicate, and the stability constants were reproduced to limits of error which were much less than the deviation of some of the points from the least-squares line. For example, the stability constants obtained from three studies at 25.0° are 11.9 M^{-1}, 11.1 M^{-1}, and 11.2 M^{-1}. The results of two studies at 15.0° are 10.5 M^{-1} and 10.6 M^{-1}. The results of three studies at 35.0° are 9.1 M^{-1}, 9.7 M^{-1}, and 9.4 M^{-1}. That the stability constant-temperature data do not appear to fit a van't Hoff relationship may be an indication of a change in the geometry of the substrate or the complex with

*The data for the trans ester were obtained by Mr. J. L. Cohen.

TABLE VII

Apparent Solubility of Methyl cis-Cinnamate at Various Theophylline Concentrations as a Function of Temperature

Theophylline ($M \times 10^2$)	Me cis-Cinnamate ($M \times 10^3$)		
	35°	15°	2°
0.00	5.40	5.05	4.93
0.35	--	5.14	5.29
0.70	--	5.38	5.58
1.05	5.92	5.54	5.72
1.40	6.14	5.72	5.98
1.75	6.31	5.99	5.82
2.10	6.47	6.09	5.82
2.45	6.64	6.23	5.82
2.80	6.79	6.38	5.88
3.15	6.95	6.08	5.88
3.50	7.16	6.08	6.09

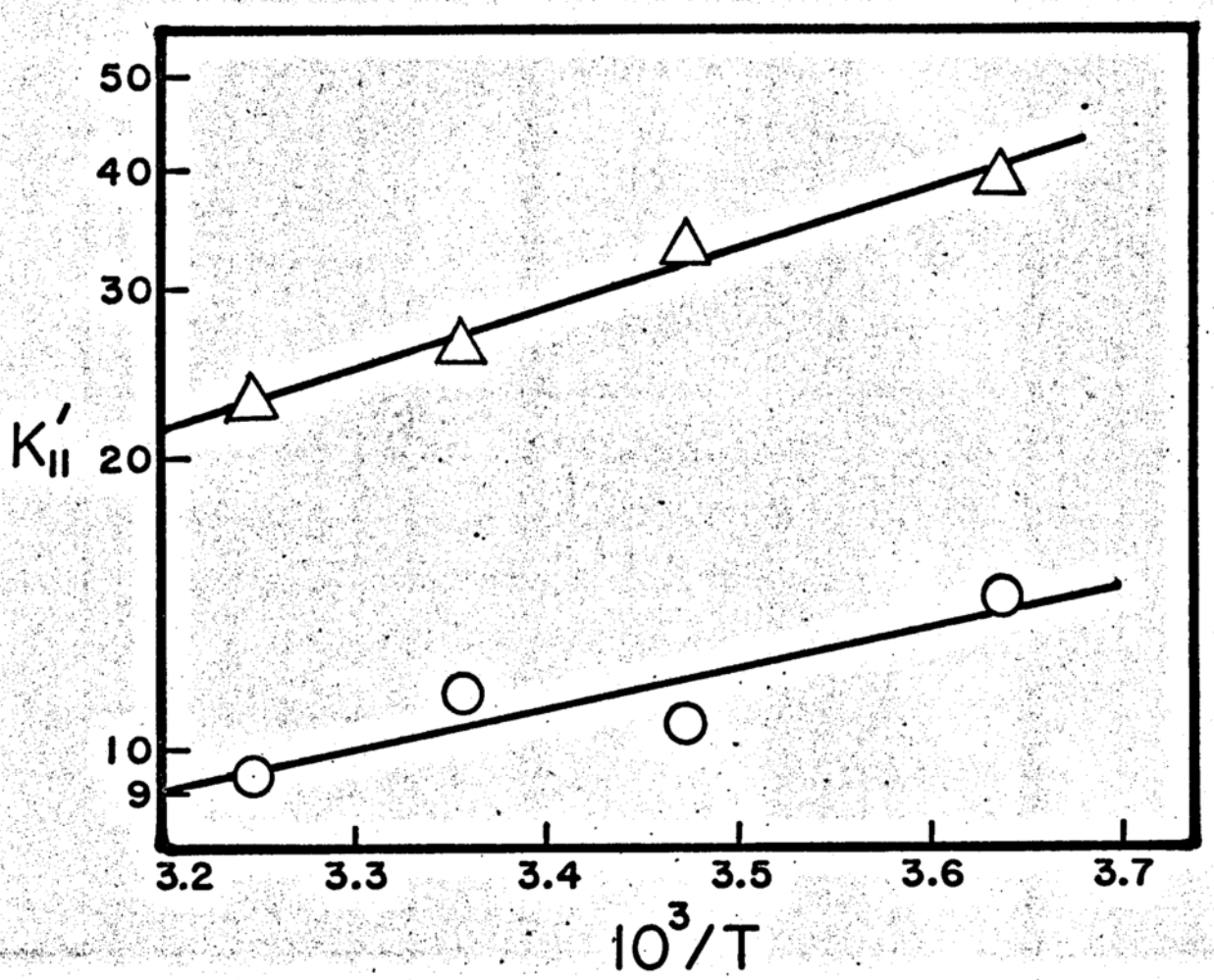


Figure 4. Plot of log K_{11}' vs. the reciprocal of the absolute temperature for methyl cis-cinnamate (circles) and methyl trans-cinnamate (triangles).

changing temperature. However, such a conclusion cannot be fully justified from the available data.

For purposes of comparison, a least-squares line was drawn through the points for the cis compound and the thermodynamic values were computed for both the cis and the trans esters. An average ΔH° was calculated from the slope of the log K_{11}' vs. 1/T for each compound. The values of ΔG° and ΔS° were then calculated for each experimental point; see Table VIII.

Theophylline was observed to cause an apparent shift in the spectrum of methyl cis-cinnamate. The data from the spectral study are plotted in Figure 5. The apparent stability constant calculated from either line is 16 M^{-1} with a probable experimental uncertainty of 30%. That the stability constants obtained from results at two different wavelengths agree might be taken as evidence for 1:1 stoichiometry. However, when we compare the stability constant of 16 M^{-1} with a K_{11}' of 11 M^{-1} obtained by the solubility method, combined with the possibility of curvature in the solubility plot and the anomalous behavior of the solubility-temperature studies, it becomes difficult to conclude that a single 1:1 complex is responsible for the methyl cis-cinnamate-theophylline interaction. On the other hand, our experience with these systems has shown that stability constants can rarely be determined from kinetic or spectral data with a precision greater than $\pm$30%. The constants obtained for this system range from

TABLE VIII

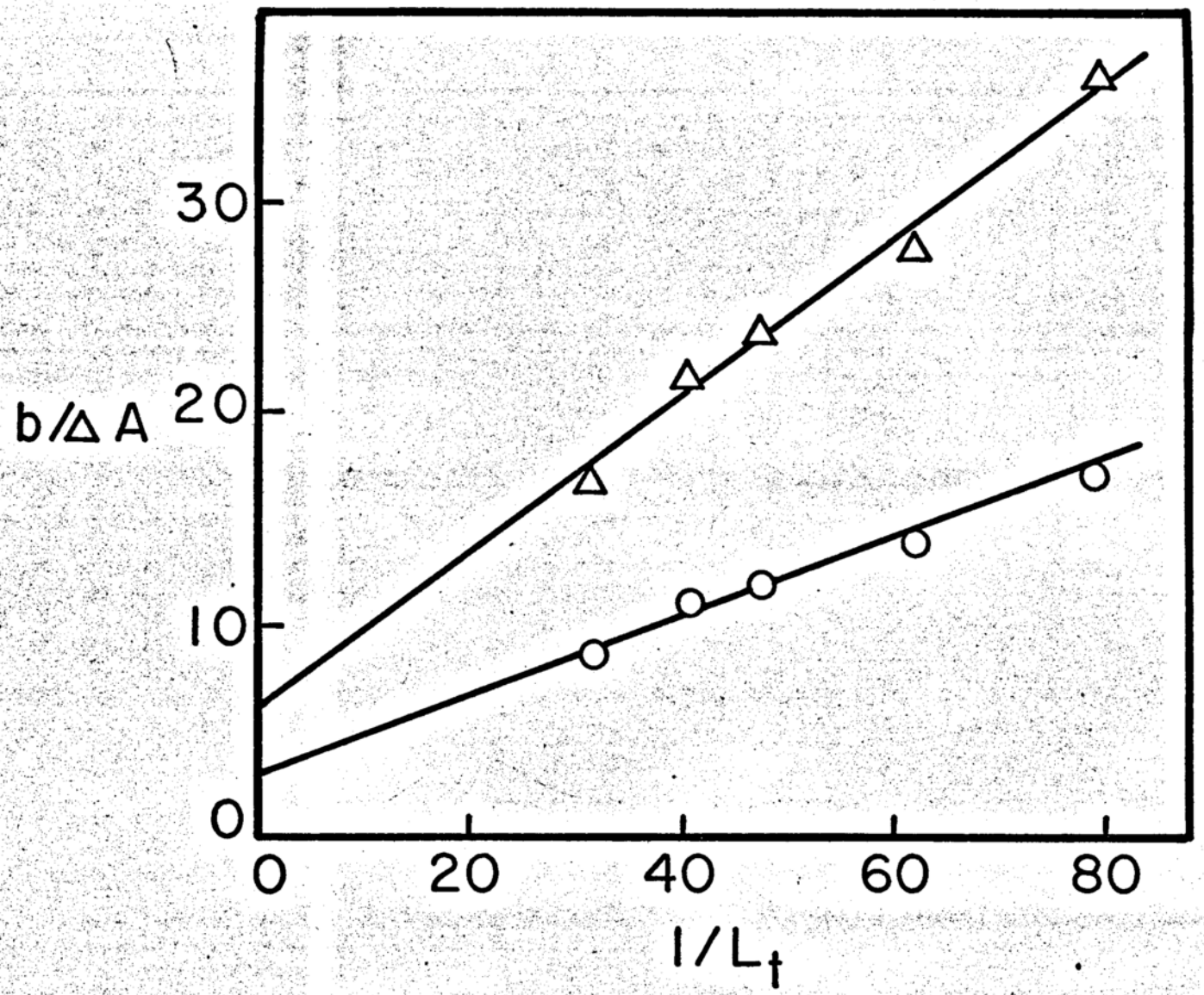
Thermodynamic Values for Complex Formation in the Methyl cis-Cinnamate- and Methyl trans-Cinnamate-Theophylline Systems

t (°C)	$K_{11}'(M^{-1})$	$\Delta G°$ (cal/mole)	$\Delta S°$ (cal/mol-deg)	$\Delta H°$ (cal/mole)
		Methyl cis-Cinnamate[a]		
2.0°	14.4	-1456	-1.75	
15.0°	10.6	-1351	-2.04	
25.0°	11.4	-1438	-1.68	-1938
35.0°	9.4	-1370	-1.84	
		Methyl trans-Cinnamate[b]		
2.0°	39.4	-2010	-2.99	
15.0°	33.2	-2005	-2.87	
25.0°	26.1	-1934	-3.01	-2867
35.0°	22.9	-1918	-2.96	

[a] pH 6.4 phosphate buffer, $\mu = 0.3$.

[b] pH 6.4 phosphate buffer, $\mu = 0.3$, 1% CH_3CN.

Figure 3. Plots of spectral data for the methyl cyanomate-diphenylfluorene system; 25.0°, 2.2×10^{-4} M CH_3CN, ester conc. 8.3×10^{-4} M, O- 2 cm cell, 314 mμ; △- 5 cm cell, 320 mμ.


30
20
10
0
b/Δ A
0
20
40
60
80
1/L_t

11 M^{-1} to 16 M^{-1}. Thus the stability constant could be described by 13.5 ± 2.7 M^{-1}, the range here being $\pm 20\%$. Since we are interested in the values of the apparent 1:1 stability constants relative to methyl trans-cinnamate, these disparities will not significantly alter the conclusions we will make from these results.

Preliminary data obtained by other workers in this laboratory indicated that 8-bromotheophylline anion (hereafter referred to as 8-bromotheophyllinate) interacted with cinnamate esters more strongly than did theophylline; therefore a study of the interaction between this ligand and methyl cis-cinnamate was attempted. 8-Bromotheophyllinate appeared to be stable at high alkaline pH's as determined by spectral measurements. It was found that 8-bromotheophyllinate reduced the rate of alkaline hydrolysis of methyl cis-cinnamate; Table IX lists the relative rates at various 8-bromotheophyllinate concentrations. The stability constant calculated from a plot of these data is 8 M^{-1} with a q_{11} of 1.06.

The stability constant for this system was also determined by the solubility technique. Figure 6 is a plot of the solubility data from Table X. K_{11}' determined from the least-squares line is 13 M^{-1}.

The spectrum of methyl cis-cinnamate was apparently shifted by 8-bromotheophyllinate. A spectral study was made at 318 mμ and at 322 mμ in 2 and 5 cm cells, respectively. The data are plotted in Figure 7. From the

TABLE IX

Relative Rates of Methyl cis-Cinnamate Alkaline Hydrolysis as a Function of 8-Bromotheophyllinate Concentration

8-Bromotheophyllinate ($M \times 10^2$)	k_s'/k_s [a]
1.2	0.933, 0.920
1.5	0.854, 0.890
2.5	0.807, 0.830, 0.825
3.5	0.807, 0.775
5.0	0.733, 0.724

[a] Second-order rate constants based on hydroxide-ion activity; 25.0°, pH 11.3 phosphate buffer, $\mu = 0.3$, 1% CH_3CN, $k_s = 5.81 \times 10^{-2} M^{-1} sec^{-1}$, ester concentration = $2.7 \times 10^{-4} M$.

TABLE X

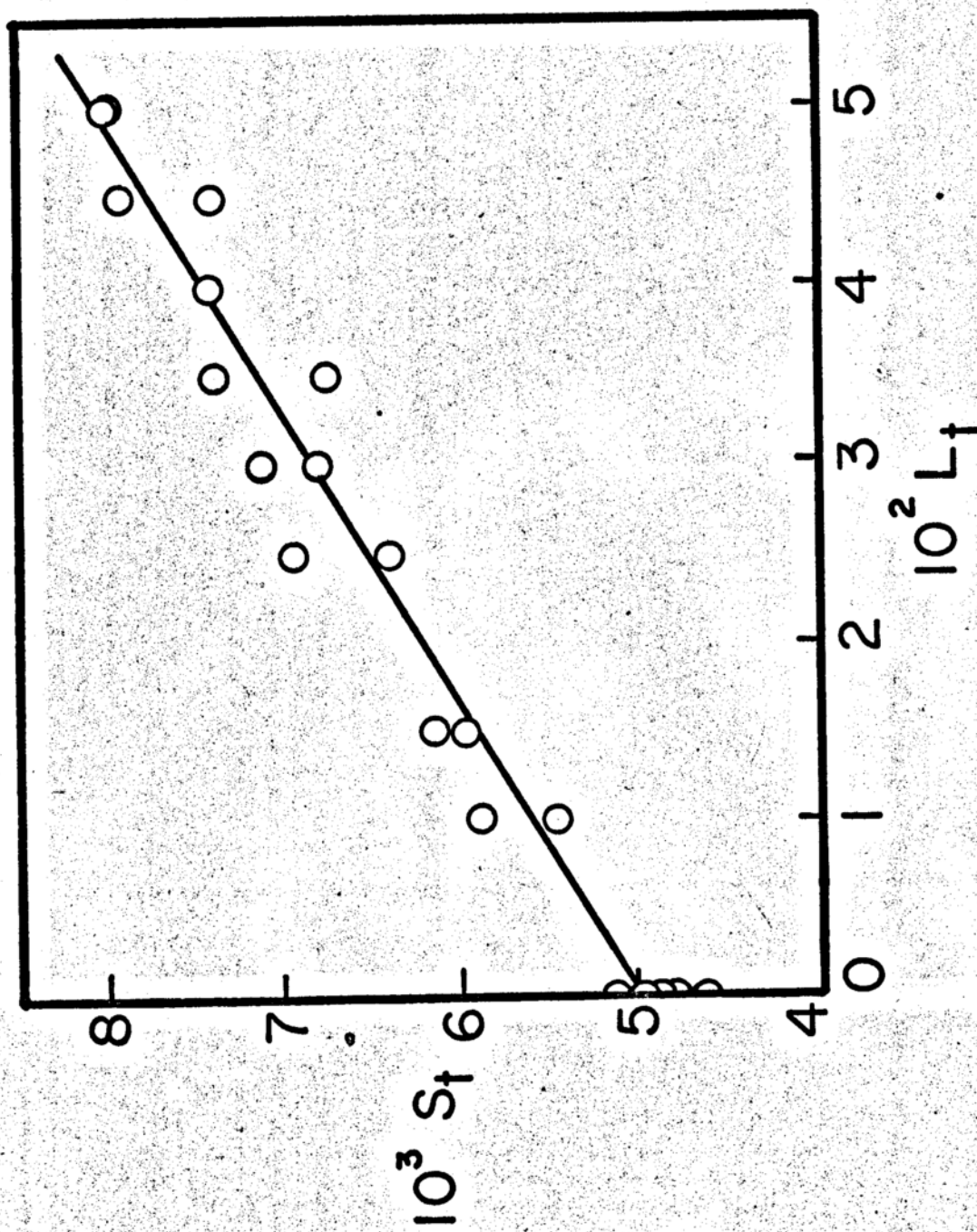
Apparent Solubility of Methyl cis-Cinnamate at Various 8-Bromotheophyllinate Concentrations[a]

8-Bromotheophyllinate ($M \times 10^2$)	Methyl cis-Cinnamate ($M \times 10^3$)
0.0	5.18[b]
1.0	5.89, 5.47
1.5	6.15, 5.98
2.5	6.40, 6.93
3.0	6.79, 7.11
3.5	6.74, 7.37
4.0	7.41
4.5	7.39, 7.91
5.0	7.98, 7.97

[a] 0.05 M pH 7.8 phosphate buffer, 25.0°, $\mu = 0.3$.

[b] Mean value from Table III.

Figure 6. Apparent solubility of methyl cis-cinnamate as a function of 8-bromocoophyllidine concentration. pH 7.8 phosphate buffer, $\mu = 0.3$, 25.0°.


$10^3 S_t$
8
7
6
5
4
0
1
2
3
4
5
$10^2 L_t$

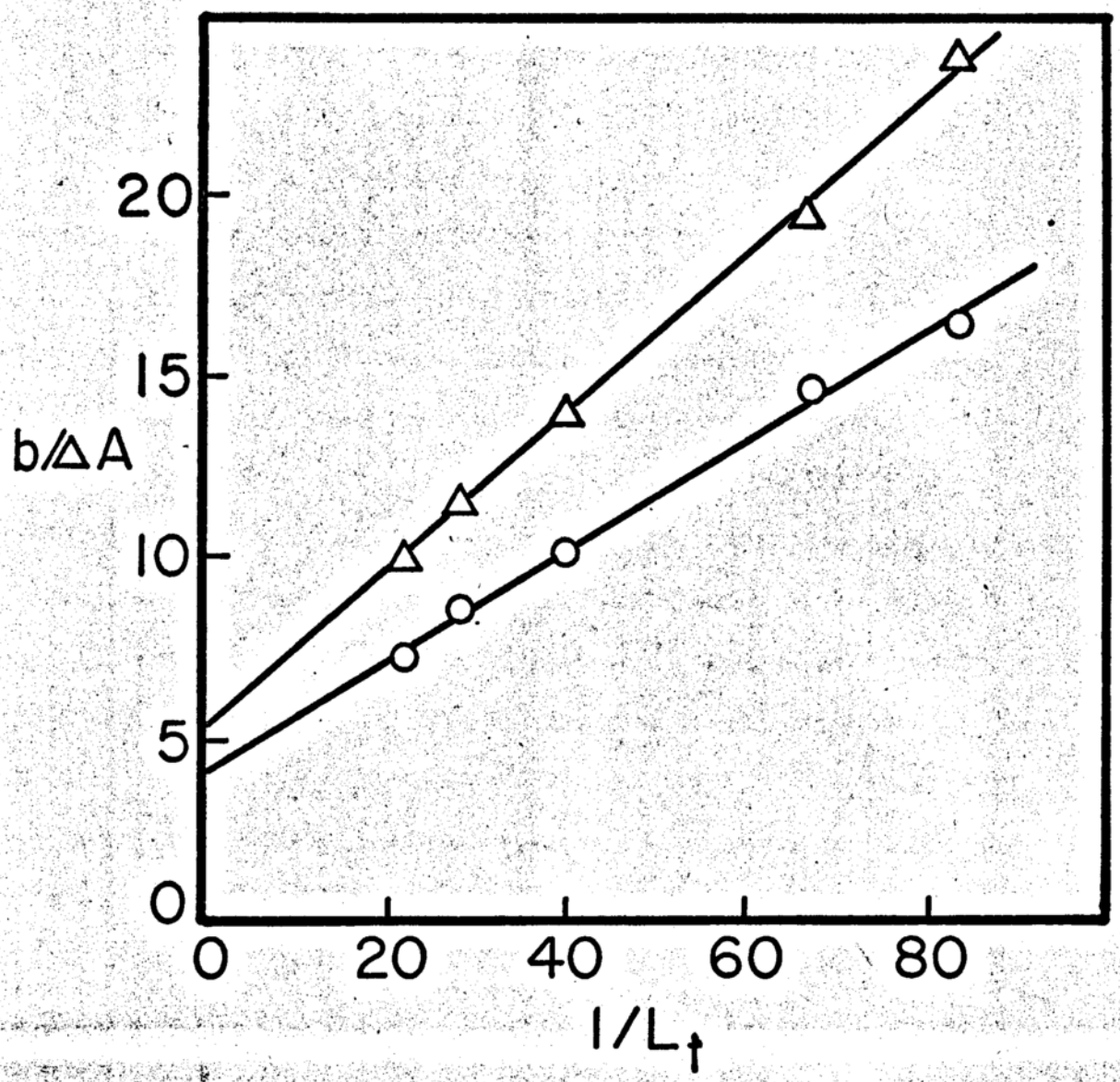


Figure 7. Plot of spectral data for the methyl cis-cinnamate-8-bromotheophyllinate system; 0.05 M pH 7.8 phosphate buffer, μ = 0.3, 1% CH_3CN, 25.0°, ester concentration 8.3×10^{-4} M. ○ - 2 cm cell, 318 mμ; △ - 5 cm cell, 320 mμ.

data at 322 mμ is computed a K_{11}' of 24 M^{-1}, while that at 318 mμ gives a K_{11}' of 27 M^{-1}. Agreement between these two values is considered reasonable in view of the uncertainty in such a determination. However, the disagreement between the solubility K_{11}' of 13 M^{-1}, the kinetically determined K_{11}' of 8 M^{-1}, and the spectral K_{11}' of 24–27 M^{-1} cannot be attributed to experimental error and is probably evidence for the existence of multiple complexes in this system (44).

Preliminary data obtained in this laboratory on 8-nitrotheophylline anion indicated that it interacts with cinnamates more strongly than do theophylline or 8-bromotheophyllinate. This compound was reported to have a pK_a of 3.58 at 25° (46). It was found to be stable at pH 11.6 for one week as indicated by spectral measurements. The anion has a λ_{max} at 387 mμ and a minimum at 303 mμ. A disadvantage to the use of this compound is that its solubility (as the anion) in pH 6.4 phosphate buffer, ionic strength 0.3, and 25.0° is relatively low, being approximately 1.5×10^{-2} M. A solubility study was conducted on the methyl cis-cinnamate-8-nitrotheophyllinate system and a K_{11}' of 18 M^{-1} was obtained. An 18% increase in the apparent solubility of methyl cis-cinnamate was observed at a maximum ligand concentration of 1.2×10^{-2} M. A preliminary calculation based on a K_{11}' of 18 M^{-1} and $q_{11} = 1$ indicated that a 22% decrease in the rate of alkaline hydrolysis of methyl cis-cinnamate

should be observed at a concentration of 1.2×10^{-2} M 8-nitrotheophyllinate. No such decrease was observed, however, when a kinetic study was attempted on this system. This may be an indication that q_{11} was not one as assumed. Because of its low solubility and the small inhibitions observed during the kinetic measurements, this ligand was not used in further studies.

B. Methyl Benzoate.--Methyl benzoate ($C_6H_5COOCH_3$), was selected for study in an effort to discover what effects the double bond has on the complexing tendencies of the cinnamoyl function. This compound represents two changes in the cinnamoyl portion of methyl trans-cinnamate. One is removal of the double bond, and the other is reduction of the distance between the carbonyl group and the ring while maintaining conjugation between the two. It was observed that methyl benzoate exhibited second-order kinetics under conditions of alkaline hydrolysis in aqueous buffers at 25.0°. The reaction was followed under pseudo-first order conditions in the spectrophotometer cell at 235 mμ. Imidazole appeared to inhibit the rate of hydrolysis as observed under pseudo-first order conditions at 282 mμ. Table XI presents some typical data for this system, and Figure 8 is a plot of the kinetic data. The stability constant calculated from this plot is 0.9 M^{-1} with a 30% uncertainty. Nine kinetic-complexation studies were conducted on the imidazole

TABLE XI

Apparent Second-Order Rate Constants for the Alkaline Hydrolysis of Methyl Benzoate in the Presence of Imidazole[a]

Imidazole (M)	$10^2 k_s'$ ($M^{-1}sec^{-1}$)
0.00	9.47
0.10	8.69
0.15	8.53
0.20	8.03
0.25	7.81
0.35	7.15
0.50	6.75

[a] 25.0°, pH 11.1 phosphate buffer, μ = 0.3, 0.25% CH_3CN, ester concentration = 7.7×10^{-4} M, based on hydroxide-ion activity.

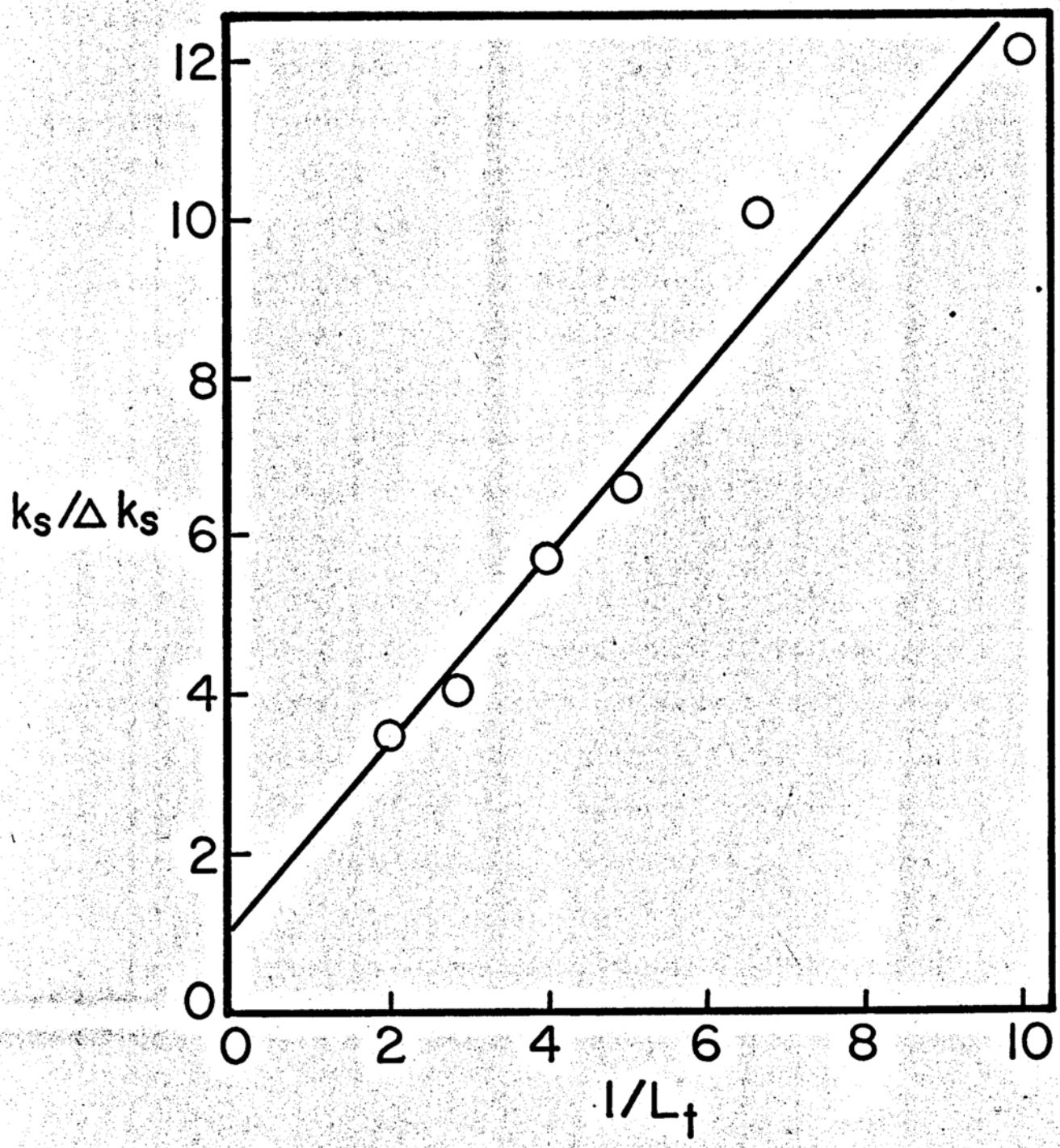


Figure 8. Plot of kinetic data for the methyl benzoate-imidazole system. (See Table XI for conditions.)

system under slightly varying conditions of acetonitrile concentration, ionic strength, and ester concentration. The stability constant evaluated from these nine runs, expressed as a mean value ±one standard deviation, is $0.91 \pm 0.30\ M^{-1}$. Acetonitrile at 0.50 and 0.35 M concentrations caused 18% and 15% diminution, respectively, in the alkaline hydrolysis rate, while imidazole at the same concentrations gave inhibitions of 24% and 19%. The relative magnitudes of these inhibitions suggest that imidazole may be acting by some mechanism besides an activity coefficient effect, though the difference between the effects of acetonitrile (taken as a non-specific medium effect) and imidazole is certainly not striking.

Theophylline anion was observed to inhibit the alkaline hydrolysis rate of methyl benzoate; Table XII and Figure 9 present the data for this inhibition. The K_{11}' computed from Figure 9 is .16 M^{-1} with an estimated 30% uncertainty. There is about a 45% decrease in the rate at 0.075 M theophyllinate concentration.

Theophylline appeared to increase the solubility of methyl benzoate in aqueous buffer; however, no success was obtained in plotting the data on a solubility isotherm. The solubility data behaved in a very irregular manner; it was not possible to obtain an accurate equilibrium solubility in buffer, so the theophylline was not the cause of the irregularities. Regardless of the unpredictable solubility data there was no doubt that theophylline

TABLE XII

Apparent Second-Order Rate Constants for the Alkaline Hydrolysis of Methyl Benzoate at Various Theophyllinate Concentrations[a]

Theophyllinate	$10^2 k_s'(M^{-1}sec^{-1})$
0.0	9.47
2.0	7.60
2.5	7.32
3.5	6.60
5.0	5.97
7.5	5.25

[a] pH 11.1 phosphate buffer, 25.0°, μ = 0.3, 0.25% CH_3CN, ester concentration = 3.8×10^{-4} M.

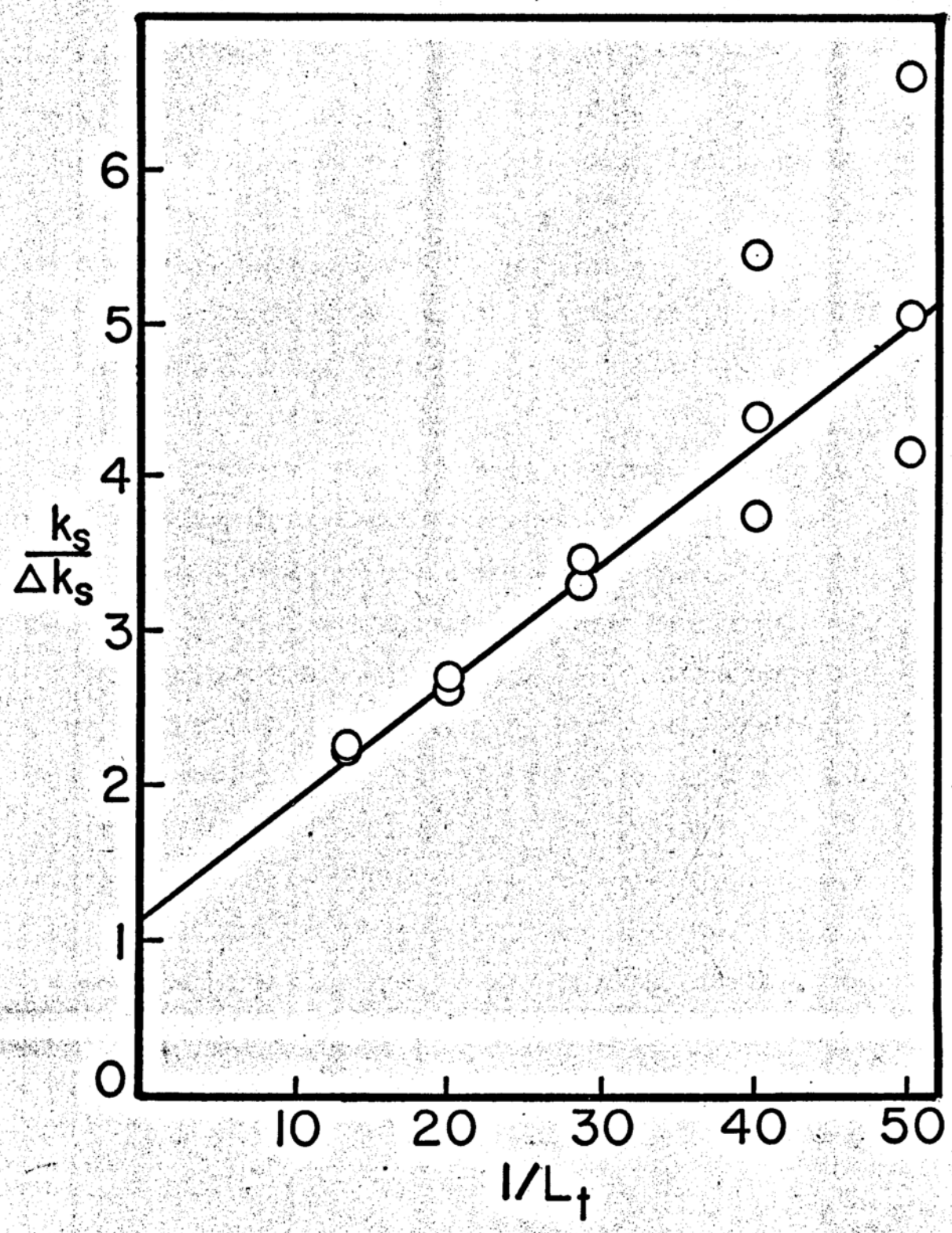


Figure 9. Plot of kinetic data for the methyl benzoate-theophyllinate system. (See Table XII for conditions.)

did increase the solubility of methyl benzoate, but no reliable stability constant could be obtained from the data. Unfortunately it was also not possible to study the methyl benzoate-theophylline system by the spectral technique because theophylline did not appear to cause a shift in the spectrum of methyl benzoate at analytically useful wavelengths. This cannot be construed as evidence for no interaction, as we shall see in the discussion of methyl p-nitrocinnamate.

We observed that 8-bromotheophyllinate reduced the rate of alkaline hydrolysis of methyl benzoate; Table XIII and Figure 10 present the data for this system. The significance of such a curve has not been described, but it is probably an indication of non-1:1 stoichiometry. Connors and Mollica have shown that the kinetic double reciprocal plot is linear if the system contains two 1:1 complexes of different reactivity (44). The methyl benzoate-8-bromotheophyllinate system certainly does not fit this situation, as seen by the curvature in Figure 10. These workers have also shown that for an S_2L system, the apparent rate constant may vary with time because of its dependence on substrate concentration. The rate data for this system were intentionally plotted over a larger time interval (three half-lives) than that routinely used to observe if k_s' changed with time. The first-order plots were, however, linear over this interval, and therefore we believe that this is probably not an S_2L system. The

TABLE XIII

Pseudo-First Order Rate Constants for the Alkaline Hydrolysis of Methyl Benzoate in the Presence of 8-Bromotheophyllinate[a]

8-Bromotheophyllinate ($M \times 10^2$)	$10^4 k_{obs}(sec^{-1})$
0.0	1.862
1.2	1.49, 1.49
1.5	1.47, 1.46
2.0	1.43
3.2	1.32
4.8	1.08

[a] pH 11.32 phosphate buffer, $\mu = 0.3$, 1% CH_3CN, 25.0°, ester concentration = 4×10^{-4} M.

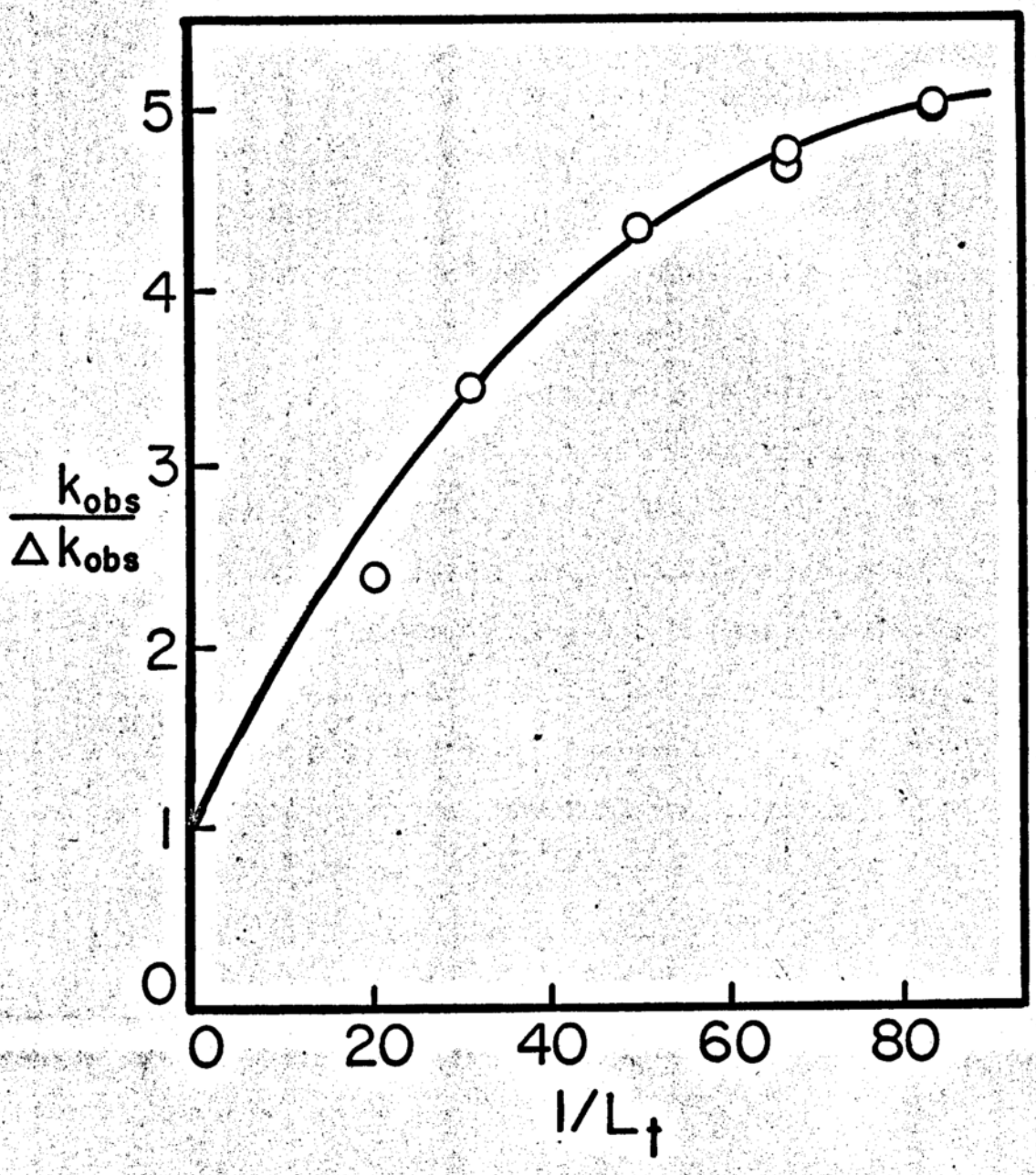


Figure 10. Plot of kinetic data for the methyl benzoate-8-bromotheophyllinate system. (See Table XIII for conditions.)

apparent linearity of the first-order plots may also eliminate most of the other possible multiple-complex systems evaluated in reference (44).

We were convinced that multiple complexes were present in the methyl benzoate-8-bromotheophyllinate system when we obtained the results of the spectral study. Although theophylline caused no shift in the spectrum of methyl benzoate, 8-bromotheophyllinate did. Studies were done in 2.0 and 5.0 cm cells, and the results are plotted in Figure 11. The stability constant evaluated at 322 mμ (triangles) is 23 M^{-1}, and that calculated from the data at 317 mμ (circles) is 13 M^{-1}. Even taking into account the possible 30% uncertainty in such a system, it is difficult to rationalize the difference between these two stability constants on any basis other than a multiple complex system. Several authors list variation of K_{11}' with wavelength as one of the criteria for classifying a system as possessing higher order complexes (44,47). The variation of K_{11}' with wavelength, along with the curve obtained from the kinetic data, certainly justifies the conclusion that this system is not described by a simple 1:1 interaction.

A solubility study was conducted on the methyl benzoate-8-bromotheophyllinate system with the hope that the shape of the isotherm might be a clue to the stoichiometry of this interaction. As with the methyl benzoate-theophylline solubility studies, however, there

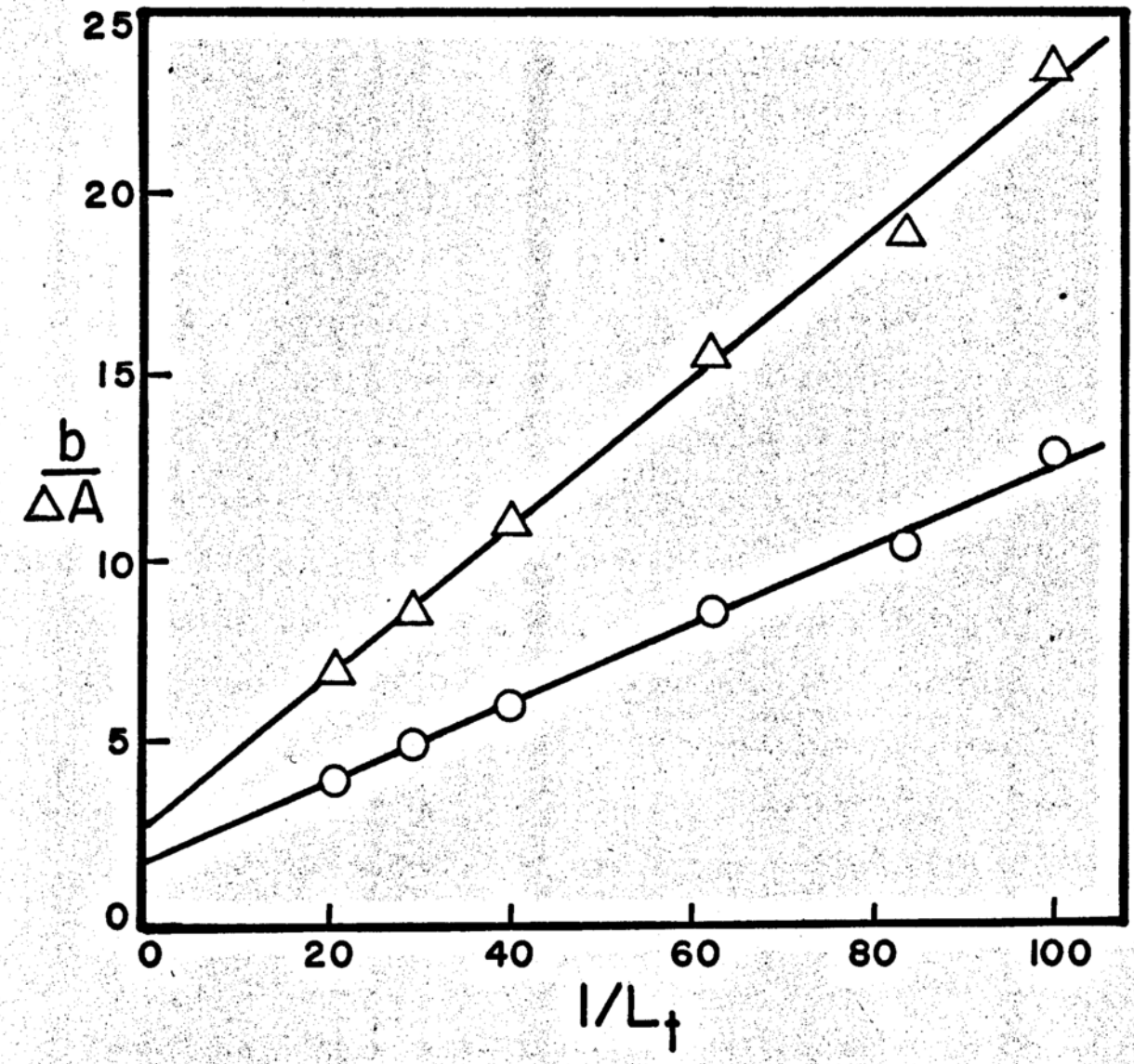


Figure 11. Plot of spectral data for the methyl benzoate-8-bromotheophyllinate system: 1% CH_3CN, 25.0°, ester concentration = 5×10^{-3} M; ○ - 2 cm cells, 317 mμ; △ - 5 cm cells, 322 mμ.

was much scatter in the data. The inconclusive nature of the data obtained in this system as well as in the methyl cis-cinnamate-8-bromotheophyllinate system led us to abandon the use of 8-bromotheophylline as a ligand for future studies.

C. Methyl Crotonate.--Methyl crotonate ($CH_3CH{=}CHCOOCH_3$) was selected for study to observe the effect that replacement of the aromatic ring with a methyl group would have on the complexation tendencies of the cinnamoyl moiety. (Methyl crotonate has the trans configuration about the double bond.) The rate of alkaline hydrolysis of this ester was studied under pseudo-first-order conditions using a manual pH-stat method (41). Imidazole appeared to inhibit the rate of hydrolysis; therefore, studies were carried out at comparable concentrations of imidazole and acetonitrile to see if solvent effects might be responsible for the observed inhibitions. Table XIV gives a comparison of the relative rates in acetonitrile and imidazole. From this comparison, which shows that imidazole in a more effective inhibitor than in acetonitrile, it seems possible that imidazole is operating through a more specific mechanism than a medium effect alone. When the data for imidazole were plotted on the usual kinetic reciprocal plot, a K_{11}' of 0.5 M^{-1} was obtained.

TABLE XIV

Relative Rates for the Alkaline Hydrolysis of Methyl Crotonate at Various Acetonitrile and Imidazole Concentrations

Acetonitrile (M)	k_s'/k_s [a]
0.25	0.910
0.50	0.856
0.75	0.833
Imidazole (M)	
0.15	0.956
0.25	0.917
0.35	0.856
0.50	0.795
0.75	0.719
1.00	0.620

[a] Second-order rate constants, pH 12.6, $\mu = 0.3$, $k_s = 3.44 \times 10^{-2}$ $M^{-1}sec^{-1}$, based on hydroxide-ion activity, ester concentration = 2.6×10^{-2} M.

The hydrolysis rate of methyl crotonate was also inhibited by theophylline anion. Rate data for this system are shown in Table XV. At a maximum theophyllinate concentration of 0.10 M a 23% decrease in the hydrolysis rate is observed. A straight line drawn through the points at high ligand concentrations on a kinetic-reciprocal plot gave a K_{11}' of 5 M^{-1}, indicating a small degree of interaction between these compounds.

Because the solubility of methyl crotonate in aqueous buffers is rather high, a solubility study was attempted using theophylline as the substrate and methyl crotonate as the ligand. Table XVI and Figure 12 present the data and the solubility isotherm. The K_{11}' calculated from the plot is 2.1 M^{-1}. Since the total increase in the theophylline solubility is only about 5% at 0.03 M methyl crotonate, it is difficult to say whether the calculated stability constant represents a real interaction or whether the solubility increase is due to solvent effects. The conclusion which can be made from this study, however, is that methyl crotonate and theophylline, if they interact at all, do so to a very small extent.

An attempt was made to study the interaction of methyl crotonate and theophylline by the spectral technique. No change in the spectrum of a 2×10^{-3} M theophylline solution was observed in a scan from 320 mμ to 295 mμ in the presence of 1×10^{-2} M methyl crotonate. As

TABLE XV

Apparent Second-Order Rate Constants for the Alkaline Hydrolysis of Methyl Crotonate in the Presence of Theophyllinate[a]

Theophyllinate ($M \times 10^2$)	$10^2 k_s'(M^{-1}sec^{-1})$
0.0	3.44
2.0	3.29
2.5	3.22
3.5	3.10
5.0	2.97
7.5	2.95, 2.91
10.0	2.66

[a] 25.0°, pH 12.6, μ = 0.3, based on hydroxide-ion activity.

TABLE XVI

Apparent Solubility of Theophylline in the Presence of Methyl Crotonate[a]

Methyl Crotonate ($M \times 10^2$)	Theophylline ($M \times 10^2$)
0.000	3.26, 3.25
0.305	3.27
0.610	3.30
0.91	3.31
1.22	3.32
1.52	3.37
1.83	3.36
2.13	3.41
2.44	3.44
2.74	3.44
3.05	3.44

[a] 25.0°, pH 6.4 phosphate buffer, $\mu = 0.3$.

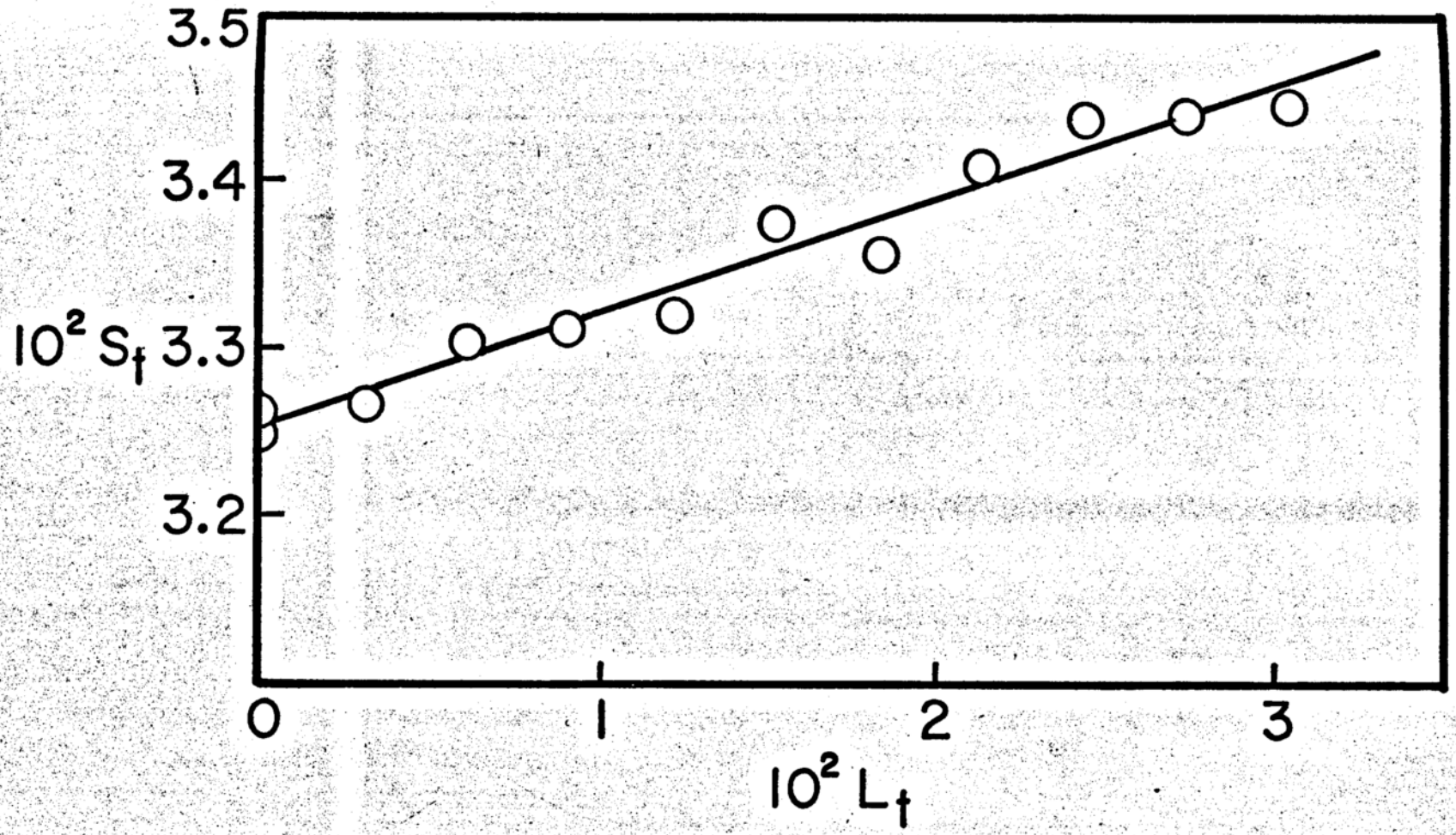


Figure 12. Plot of apparent solubility of theophylline as a function of methyl crotonate concentration. (See Table XVI for conditions.)

previously mentioned, this is not necessarily an indication of no interaction.

D. Methyl Acetate.--Methyl acetate, CH_3COOCH_3, represents the extreme in modification of the cinnamoyl portion of methyl trans-cinnamate. Here we have complete replacement of the cinnamoyl group with an acetyl group, which contains no π-electron system. Since methyl crotonate appeared to interact to a small extent with imidazole and theophylline, it was necessary to assure ourselves that this interaction was not a nonspecific one which any methyl ester could undergo.

The alkaline hydrolysis of methyl acetate was studied under pseudo-first order conditions by the pH-stat method. The effects of imidazole, acetonitrile, and theophylline anion on the rate were measured; the data are presented in Table XVII and Figure 13. It is obvious from these data that imidazole and acetonitrile are affecting the hydrolysis rate to about the same extent. Whether both compounds are operating by the same mechanism, i.e., a medium (activity coefficient) effect, is difficult to determine; and a more detailed discussion of this will be presented in another section of this thesis.

E. Cinnamamide and Benzamide.--Nakano and Higuchi have recently published the results of a study concerning the interaction of benzamide and cinnamamide with

TABLE XVII

The Apparent Second-Order Rate Constants for the Alkaline Hydrolysis of Methyl Acetate in the Presence of Various Additives

Additive (M)	$k_s'(M^{-1}sec^{-1})^a$
0.00	0.264
Imidazole	
0.10	0.248
0.15	0.245
0.25	0.236
0.35	0.229
0.50	0.219
Acetonitrile	
0.10	0.247
0.35	0.231
0.50	0.220
Theophyllinate	
0.075	0.257

[a]Based on hydroxide-ion activity, 25.0°, μ = 0.3, pH 11.7, ester concentration = 2.35×10^{-2} M.

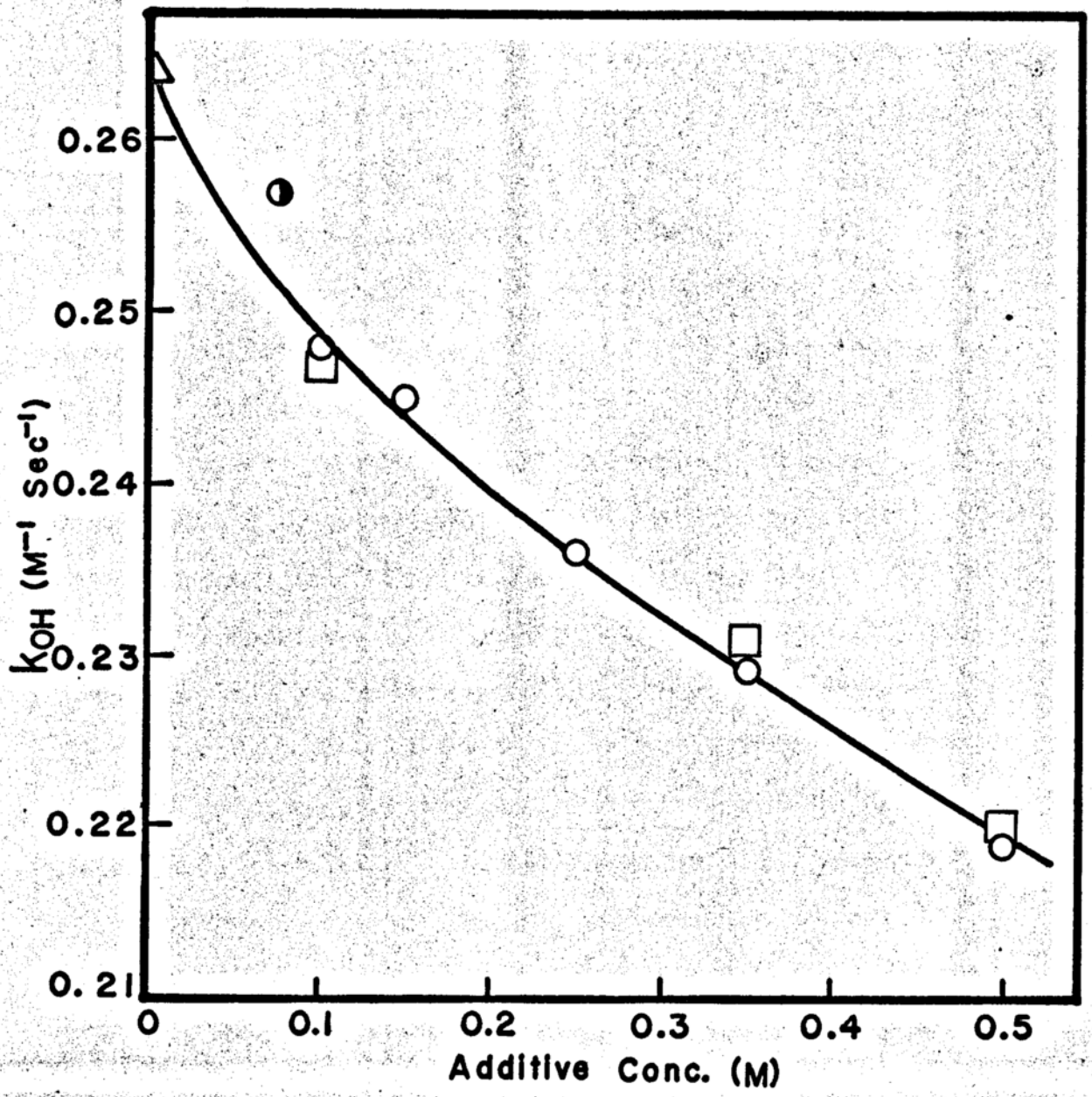


Figure 13. Apparent second-order rate constant for the alkaline hydrolysis of methyl acetate as a function of various additive concentrations (see Table XVII for conditions);
○- imidazole, □- acetonitrile,
◑- theophyllinate, △- no additive.

theophylline (14). These results indicated that theophylline and benzamide interact with an apparent stability constant of 12 M^{-1} while cinnamamide and theophylline interact with a K_{11}' of 32 M^{-1}. The results of our study on methyl benzoate and theophylline indicated that methyl benzoate interacted to about the same degree as methyl trans-cinnamate. This conclusion is based primarily on the results with theophyllinate, although the magnitude of the increase in the solubility of methyl benzoate brought about by 0.035 M theophylline indicated a degree of interaction similar to that between methyl trans-cinnamate and theophylline. The results of Nakano and Higuchi indicate that cinnamamide and theophylline interact with a stability constant nearly three times larger than that for benzamide and theophylline. It was difficult to rationalize that such subtle differences in bond angles, bond lengths, and electronic properties which exist between amides and esters of the same carboxylic acids could be responsible for such drastic differences in complexing tendency with a common ligand. Because of these discrepancies, and because Nakano and Higuchi did not present experimental details, we decided to study the interaction of theophylline with benzamide and cinnamamide under our experimental conditions. The data might provide a comparison between benzoates and cinnamates since the results of our studies with methyl benzoate and theophylline were not quantitatively reliable.

The interaction of cinnamamide and benzamide with theophylline was studied by the solubility technique. Tables XVIII and XIX and Figures 14 and 15 present the data for these systems. The stability constant for the cinnamamide-theophylline interaction calculated from Figure 14 is 28 M^{-1}. The apparent solubility of cinnamamide is increased by 57% at the maximum theophylline concentration of 0.035 M. The stability constant for the benzamide-theophylline interaction is 15 M^{-1}; the apparent solubility of theophylline is increased 55% by 0.08 M benzamide.

The spectrum of cinnamamide appeared to be shifted by theophylline. The data from the spectral study are shown plotted in Figure 16. The stability constant calculated from this line is 17 M^{-1}. The discrepancy between this value and that obtained by solubility measurements is taken as evidence for the presence of multiple complexes.

Since amides are quite resistant to alkaline hydrolysis, it was possible to employ the solubility and spectral methods to study the interaction between cinnamamide and theophyllinate. (With the less stable ester substrates it has been possible to measure interactions with theophyllinate only by the kinetic method.) The solubility and spectral techniques were applied to this system, and the data are presented in Table XX, Figure 17 and Figure 18. At theophyllinate

TABLE XVIII

Apparent Solubility of Cinnamamide in the Presence of Various Concentrations of Theophylline[a]

Theophylline (10^2M)	Cinnamamide (10^2M)
0.00	0.849[b]
1.05	1.03, 1.04
1.40	1.09
1.75	1.19
2.10	1.22, 1.24
2.45	1.31, 1.30
2.80	1.38, 1.39
3.15	1.43
3.50	1.49

[a] 25.0°, pH 6.4 phosphate buffer, μ = 0.3.

[b] Mean of seven determinations.

TABLE XIX

Apparent Solubility of Theophylline in the Presence of Various Concentrations of Benzamide[a]

Benzamide (L_t) ($M \times 10^2$)	Theophylline (S_t) ($M \times 10^2$)
0.0	3.28, 3.24
0.8	3.50
1.6	3.77
2.4	4.04
3.2	4.30
4.0	4.50
4.8	4.96
5.6	5.09
6.4	5.37
7.2	5.65
8.0	5.90

[a] 25.0°, pH 6.4 phosphate buffer, $\mu = 0.3$.

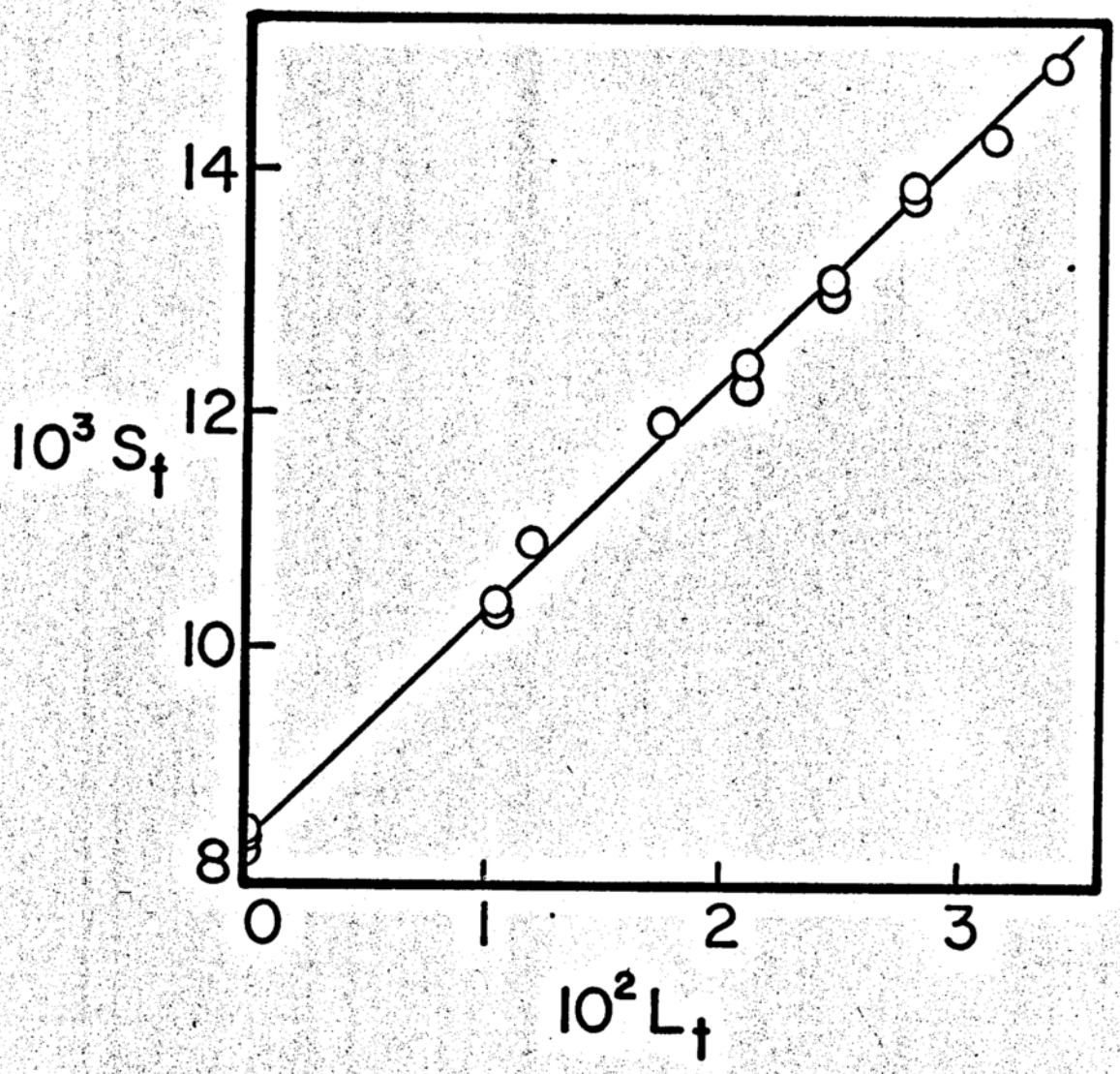
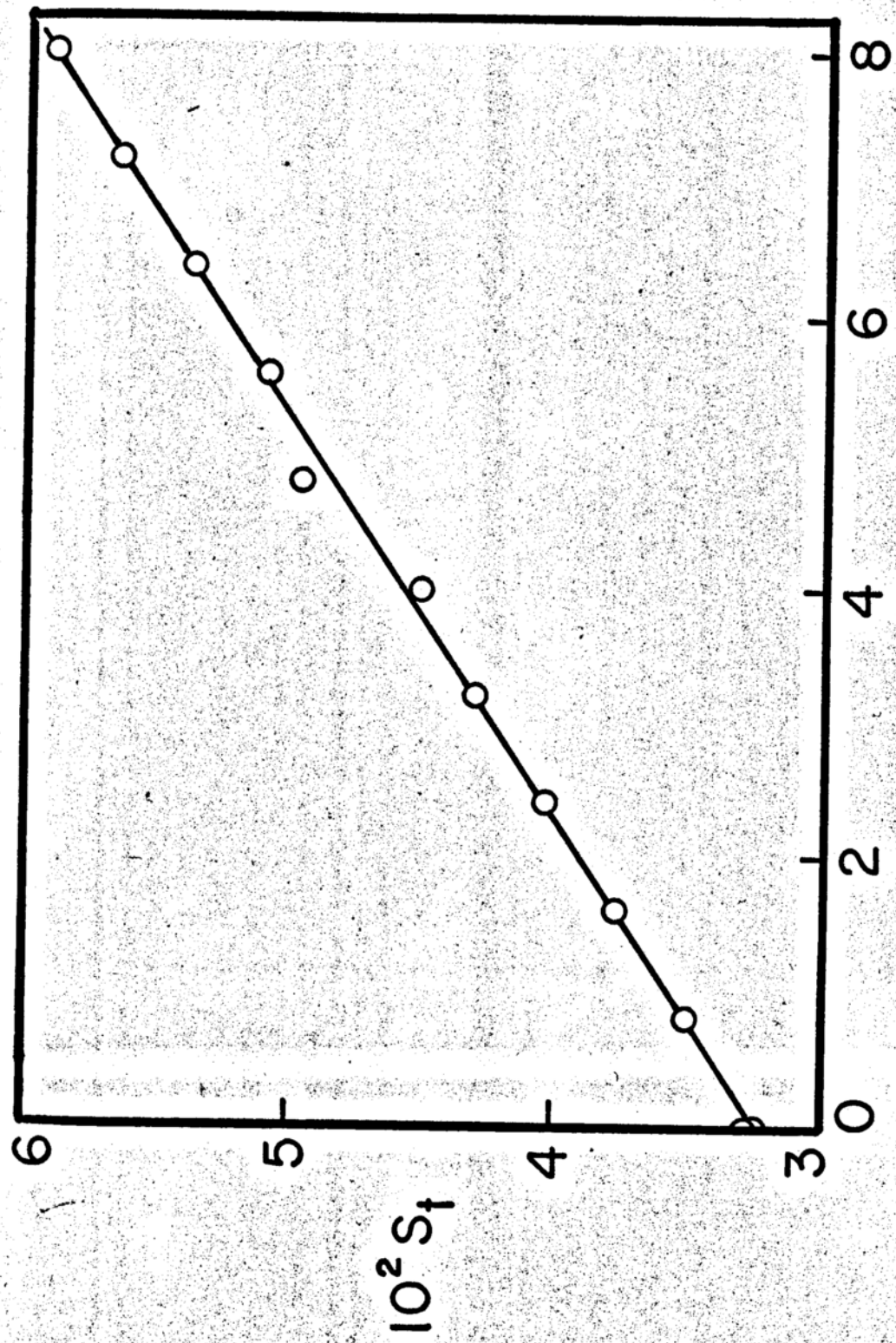


Figure 14. Plot of apparent solubility of cinnamamide as a function of theophylline concentration. (See Table XVIII for conditions.)

Figure 15. Plot of apparent solubility of theophylline as a function of benzamide concentration. (See Table XIX for conditions.)


6
5
4
3
0
2
4
6
8
$10^2 S_t$
$10^2 L_t$

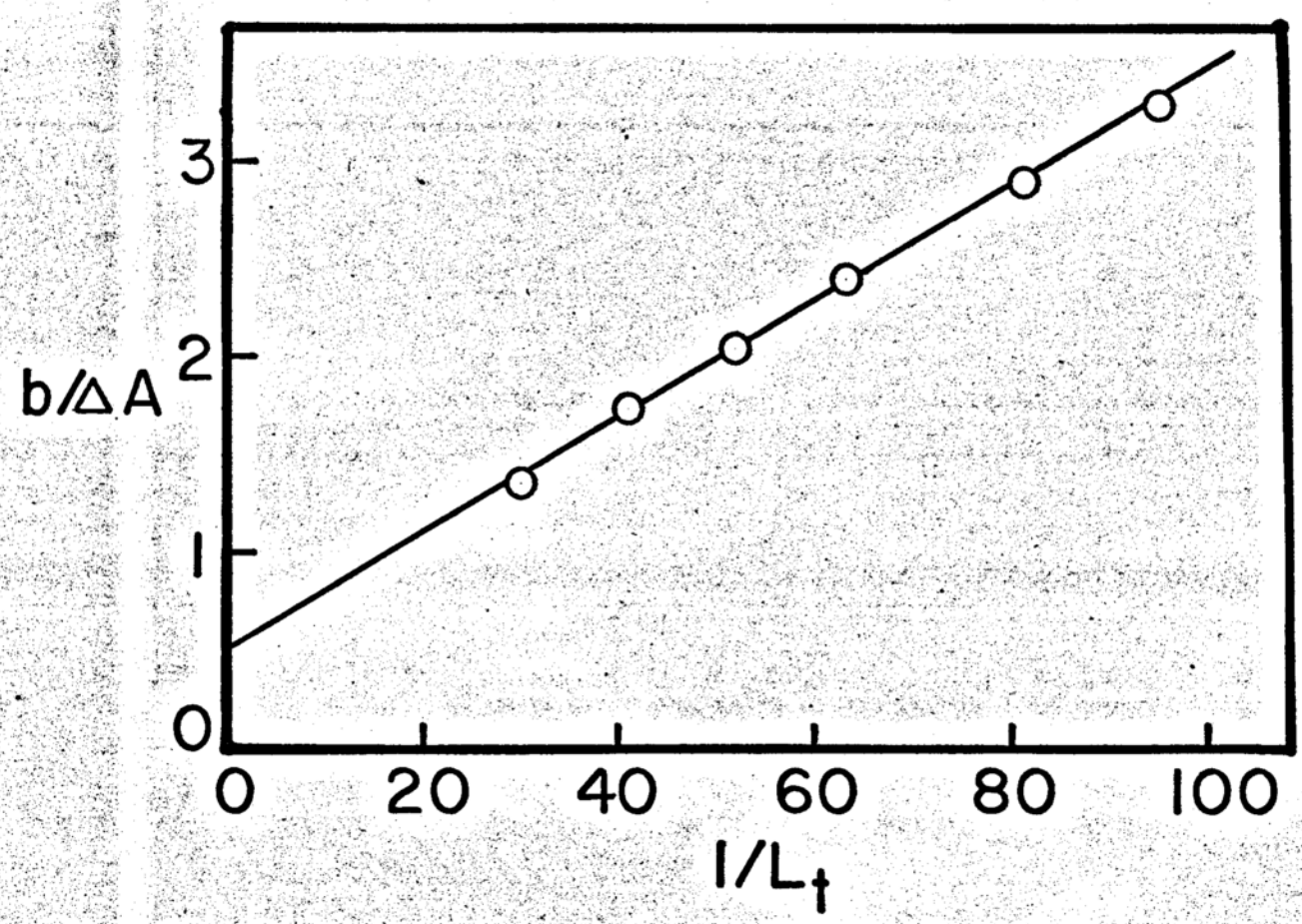


Figure 16. Plot of spectral data for the cinnamamide-theophylline system; 1% CH_3CN, 25.0°, amide concentration = 1.45×10^{-3} M, 314 mμ, 1 cm cell.

TABLE XX

Apparent Solubility of Cinnamamide in the Presence of Various Concentrations of Theophyllinate[a]

Theophyllinate (10^2M)	Cinnamamide (10^2M)
0.0	0.86
1.0	0.98
2.0	1.13
3.0	1.25
4.0	1.37
5.0	1.52
6.0	1.58
7.0	1.70
8.0	1.80
9.0	1.92
10.0	2.02

[a] 25.0°, pH 11 phosphate buffer, μ = 0.3.

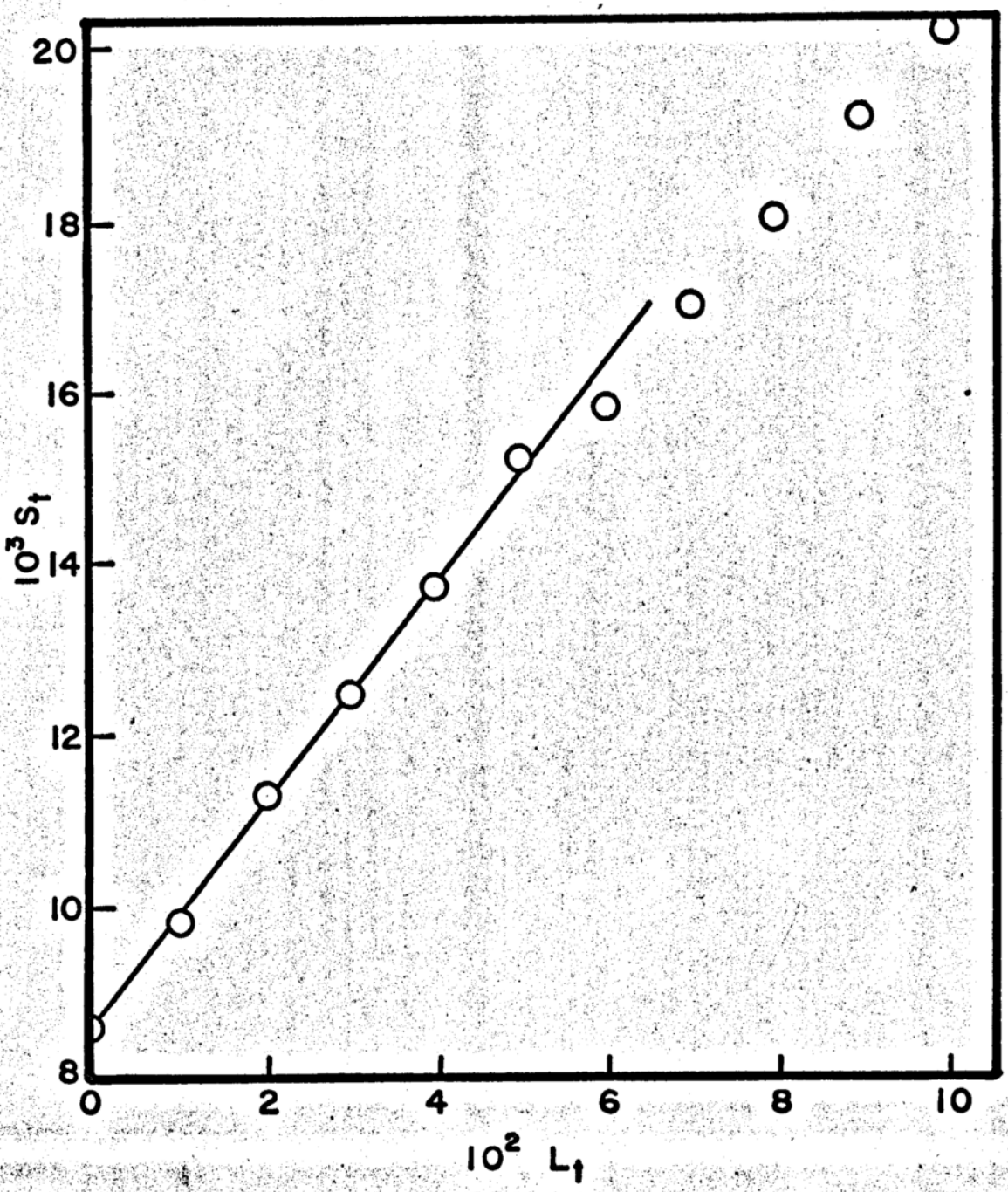


Figure 17. Plot of apparent solubility of cinnamide as a function of theophyllinate concentration. (See Table XX for conditions.)

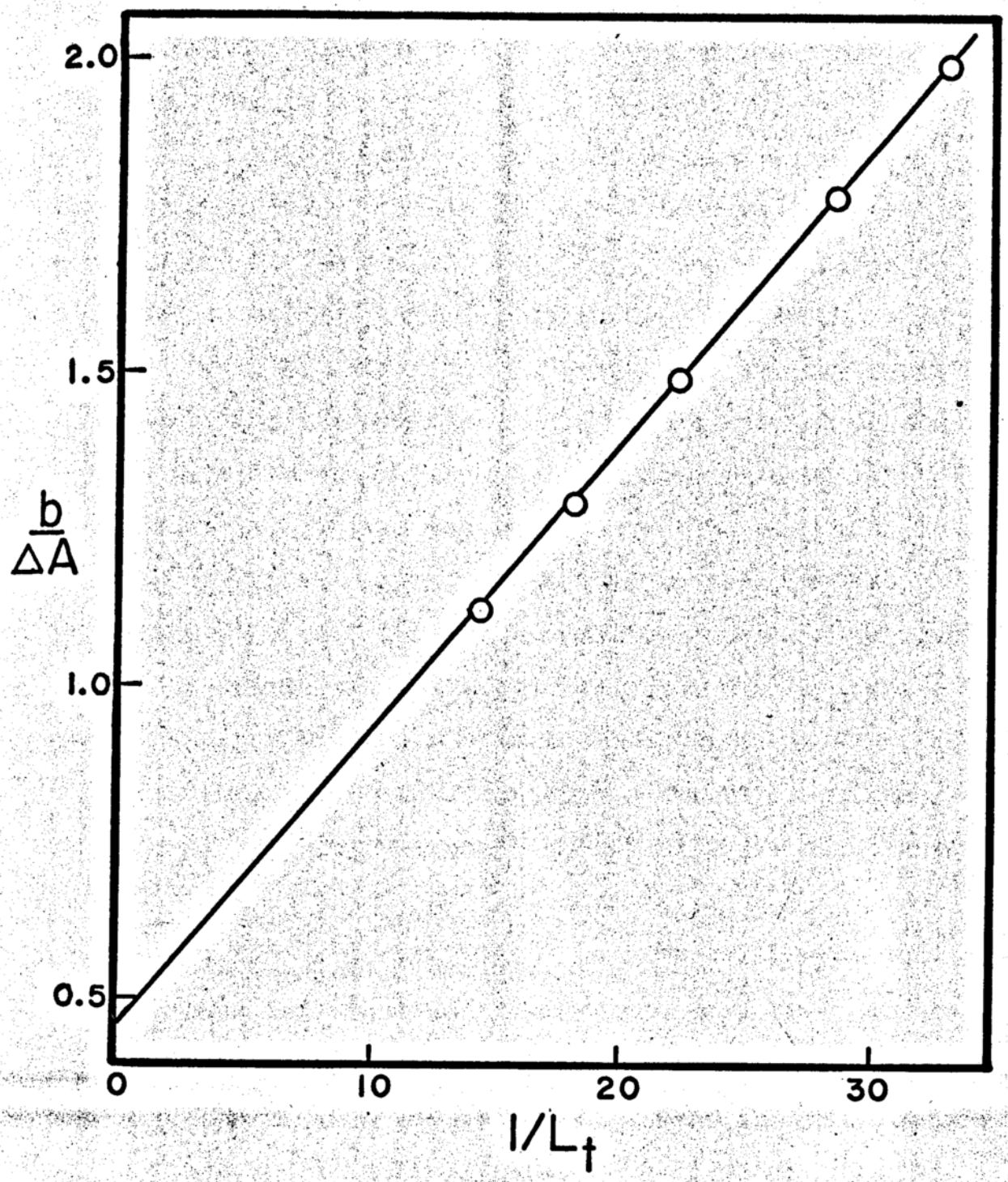


Figure 18. Plot of spectral data for the cinnamamide-theophyllinate system: 25.0°, 1% CH_3CN, 315 mμ, 1 cm cell, amide concentration ≅ 1.45×10^{-3} M.

concentrations greater than 0.05 M the solubility isotherm (Figure 17) obviously changes slope; the reason for this is not known. The initial portion is linear, however, and the stability constant computed from the line through these points is 17 M^{-1}. The stability constant calculated from the spectral plot (Figure 18) is 10 M^{-1}. The discrepancy between these two constants is larger than the experimental error for such a system, and it is taken as evidence for the existence of multiple complexes. It is interesting to note that when methyl cis- and trans-cinnamate are used as substrates, the stability constants for the interaction with theophylline are about double those for the interaction with theophyllinate. With cinnamamide and the two forms of theophylline, no simple relationship can be discerned because of the apparent existence of multiple complexes.

F. Methyl 2,6-Dichloro-trans-Cinnamate.--This compound was selected for study because it is non-planar, but the trans configuration is retained about the double bond of the side chain. The two relatively large chlorine atoms cause the side chain to be forced out of planarity with the aromatic ring. Solubility studies were attempted with this compound and theophylline, but small, irreversible, spectral shifts occurred when the ester was equilibrated with buffer. For example, a freshly prepared solution of the ester in buffer had a UV maximum at 270 mμ while

isooctane solutions of the ester had a maximum at 263 mμ. After equilibration of excess ester with buffer for 24 hours, the spectrum of the saturated solution had its maximum at 276 mμ while an isooctane extract of this solution had its maximum at 268 mμ. Numerous experiments on this system failed to discover the reason for these shifts. There was no doubt, however, that theophylline increased the solubility of this ester, and with the hope that the minor spectral shifts did not represent gross alterations in its structure, solubility studies were conducted on the ester-theophylline system. The results of one of these studies are presented in Table XXI and Figure 19. The apparent stability constant computed from this least-squares line is 39 M^{-1}. Another study duplicated this constant to within 5%.

Spectral studies were attempted on this system, but theophylline did not shift the spectrum of methyl 2,6-dichlorocinnamate in the wavelength region of analytical utility.

G. Methyl p-Nitro-trans-Cinnamate.--This ester was selected for study because it presented an opportunity to observe the effects of an electron-withdrawing ring substituent on complexation. Solubility studies were attempted on this ester in the presence of theophylline; however, several experimental difficulties were encountered. The first was an unexpected small spectral

TABLE XXI

Apparent Solubility of Methyl 2,6-Dichlorocinnamate in the Presence of Various Concentrations of Theophylline[a]

Theophylline (10^2M)	Me 2,6-Dichlorocinnamate (10^4M)
0.00	0.675, 0.721
0.35	0.713
0.70	0.844
1.05	0.986
1.40	1.09
1.75	1.15
2.10	1.30
2.45	1.36
2.80	1.54
3.15	1.51
3.50	1.56

[a] 25.0°, pH 6.4 phosphate buffer, μ = 0.3.

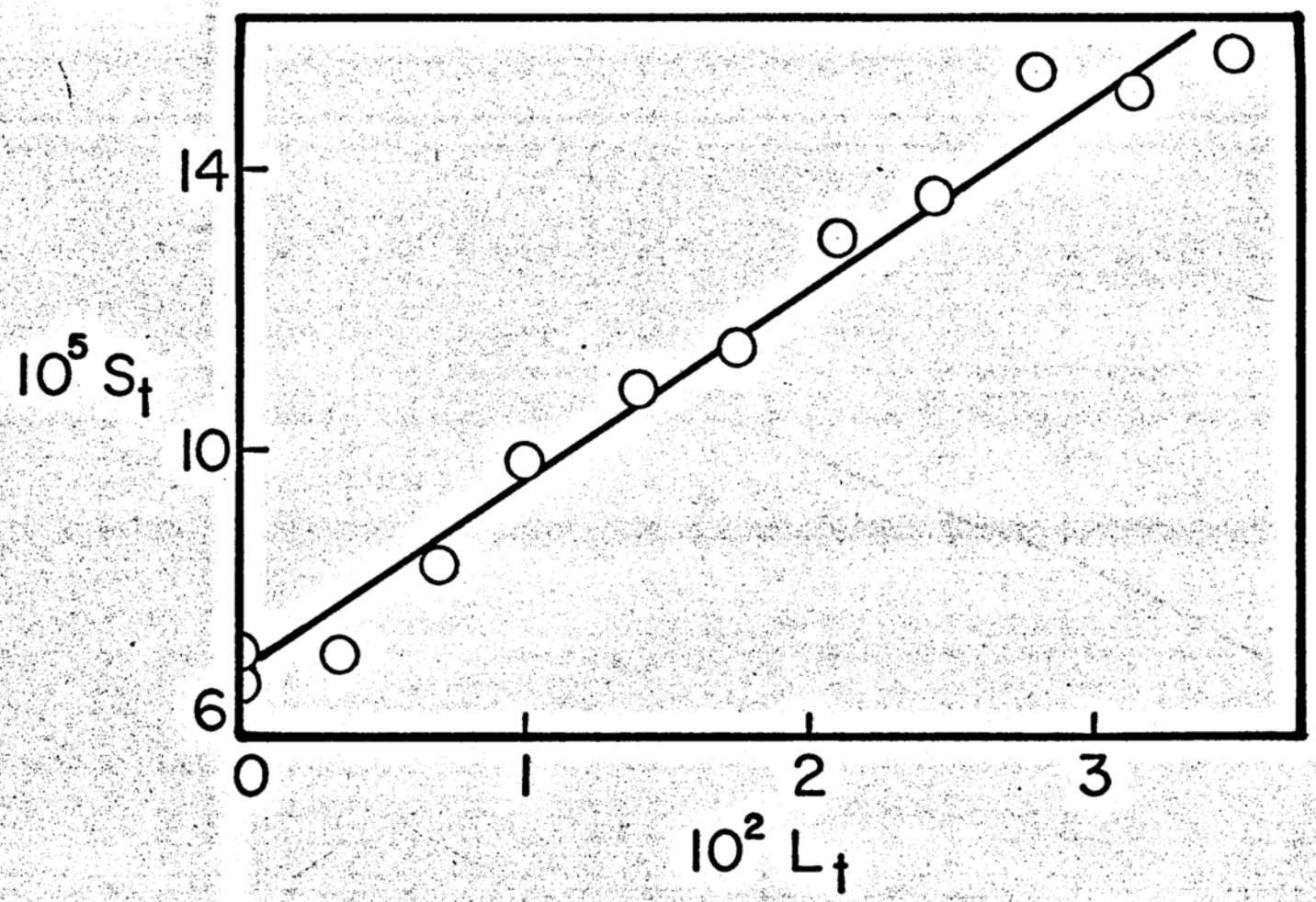


Figure 19. Plot of apparent solubility of methyl 2,6-dichlorocinnamate as a function of theophylline concentration. (See Table XXI for conditions.)

shift upon equilibration with buffer. Freshly prepared solutions of the ester had a λ_{max} at 302 mμ while those equilibrated for 24 hours or longer had a λ_{max} at 306 mμ. As with methyl 2,6-dichlorocinnamate, the reason for this shift was not apparent; therefore solubility studies were conducted with the hope that this spectral shift did not represent any gross alterations in the properties and structure of the ester. The other difficulty encountered was that abnormally long equilibration times were required for solubility studies. With most substrates, 24 hours was enough time to attain solubility equilibrium; however, this ester did not reach stable saturation concentrations in this time interval as evidenced by marked scatter in the solubility data. Even after a four day equilibration period, there was still some scatter to the data, presumably due to non-equilibration since the analytical technique used for this ester employed no extractions (which tend to introduce larger experimental errors due to multiple volumetric transfers and dilutions).*

The data from the solubility studies with methyl p-nitrocinnamate in the presence of various theophylline concentrations are presented in Table XXII and Figure 20. The values for S_t at the two highest ligand concentrations

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*Subsequent work by Miss H. Stelmach reveals that the absorbance of a solution of this ester decreases upon exposure to light, perhaps as a consequence of trans-cis isomerization; thus some imprecision in the results may be ascribable to this cause.

TABLE XXII

Apparent Solubility of Methyl p-Nitrocinnamate at Various Theophylline Concentrations[a]

Theophylline (10^2M)	Methyl p-Nitrocinnamate (10^5M)
0.00	4.85, 5.05
0.35	5.40, 5.44
0.70	5.58
1.05	6.15, 6.19
1.40	6.12, 6.10
1.75	6.97
2.10	7.03
2.45	7.63
2.80	7.88
3.15	7.85
3.50	7.63

[a] 25.0°, pH 6.4 phosphate buffer, μ = 0.3.

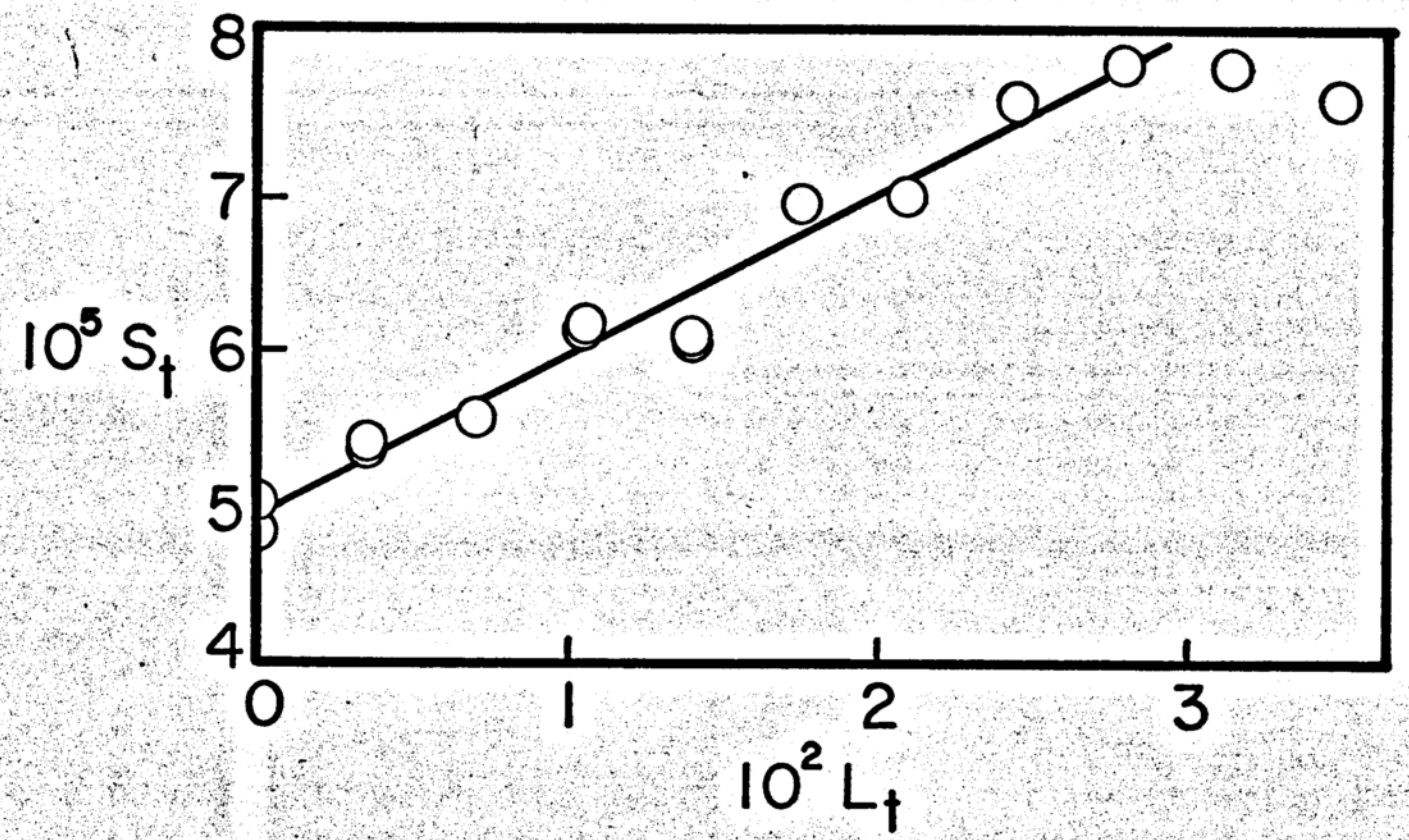


Figure 20. Plot of apparent solubility of methyl p-nitrocinnamate as a function of theophylline concentration. (See Table XXII for conditions.)

were not included in the least-squares calculations since it is quite possible that they represent formation of an insoluble complex. The K_{11}' calculated from Figure 20 is 21 M^{-1}. The structural implications of this stability constant will be discussed later.

The results of attempted spectral studies on the methyl p-nitrocinnamate-theophylline system are quite interesting. A UV scan from 350 mμ to 290 mμ of a 2×10^{-5} M ester solution in buffer produced a spectrum with a λ_{max} of 300-302 mμ. A scan of a solution containing the same amount of ester and 0.032 M theophylline produced a spectrum which was essentially identical in shape and intensities with the one in buffer from 350 mμ to 325 mμ. From 325 mμ to 302 mμ, however, the spectrum of this solution had slightly lower absorbances than the solution containing no theophylline. In addition to this, the peak was at 307-309 mμ; see Figure 21. This is the only substrate we have studied that could be scanned near the λ_{max} in the presence of theophylline. This is also the first piece of evidence that a bathochromic shift in the spectrum of the substrate occurs upon complexation, although this is an unusual compound in that the spectral shift occurs only near the λ_{max}. With previously studied substrates, spectral studies have been performed by measuring intensity differences on the long-wavelength side of a spectral band. It was never established whether these intensity

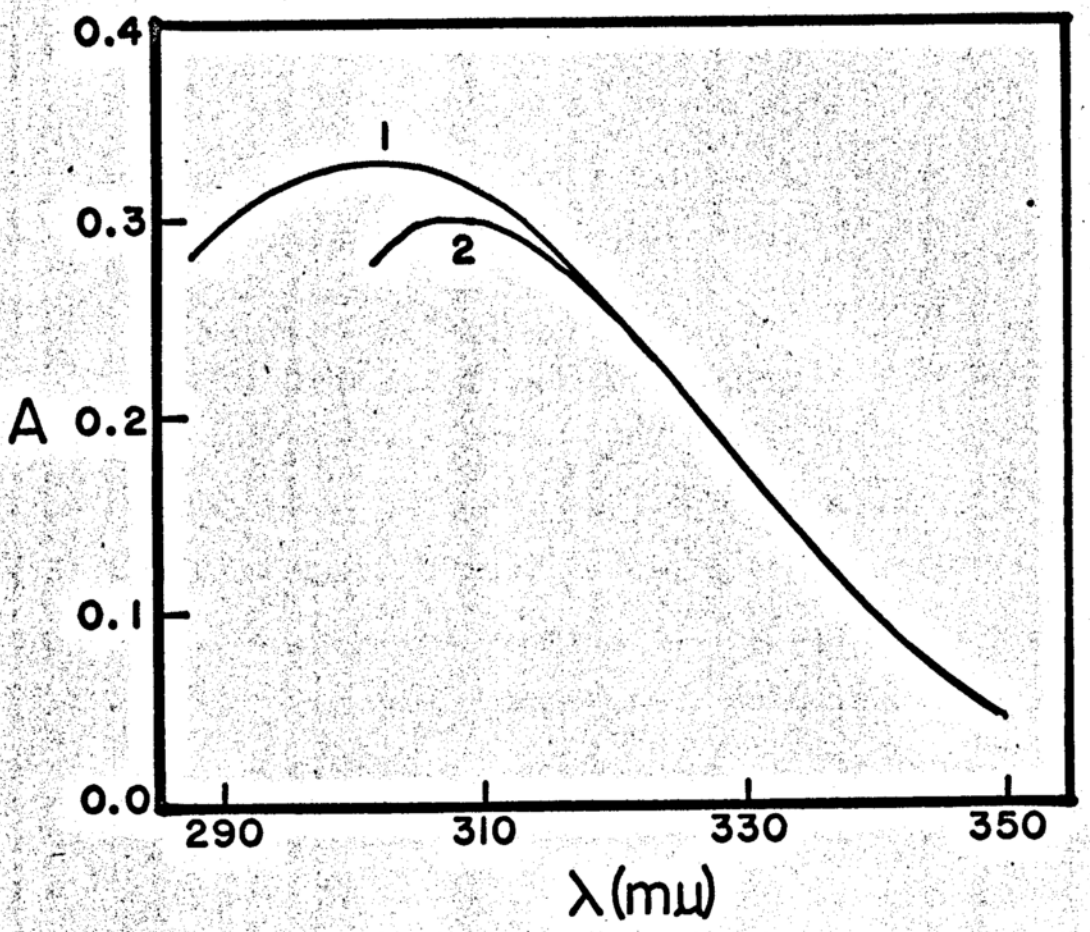


Figure 21. Plot of absorbance vs. wavelength for methyl p-nitrocinnamate. Spectrum 1: 2×10^{-5} M methyl p-nitrocinnamate in aqueous buffer, Spectrum 2: 2×10^{-5} M methyl p-nitrocinnamate - 3.15×10^{-2} M theophylline in aqueous buffer.

differences were due to bathochromic spectral shifts or absorptivity changes. There is little doubt with methyl p-nitrocinnamate that there is a bathochromic shift of λ_{max}. Unfortunately the magnitude of the absorbance change brought about by this shift is too small to be measured accurately so that it was not possible to obtain a stability constant for the interaction with theophylline by the spectral technique.

H. Methyl 1-Naphthoate and Methyl 2-Naphthoate.--These compounds were selected for study because of their similarity to benzoates, because they contain a conjugated system of aromatic rings, double bonds, and ester carbonyls similar to that of cinnamate esters, and in addition, they are relatively large, planar molecules. Solubility studies with these two compounds and theophylline as the ligand were attempted. The data are presented in Tables XXIII and XXIV and Figures 22 and 23. Notice that in both cases, the solubility of the ester is increased nearly 800% by 0.035 M theophylline. The positive curvature in solubility isotherms with increasing ligand concentration has been observed by other workers (48-50) and is ascribed to the presence of complexes containing L to a higher order than one (45). As a first approximation, the assumption was made that both systems contained SL and SL_2 complexes, and the treatment of the data as discussed in reference (45) was applied. This treatment consists of plotting the data

TABLE XXIII

Apparent Solubility of Methyl 1-Naphthoate at Various Theophylline Concentrations[a]

Theophylline (10^2M)	Methyl 1-Naphthoate (10^3M)
0.000	0.320, 0.340
0.350	0.444
0.700	0.579
1.05	0.750
1.40	0.923
1.75	1.11
2.10	1.30
2.45	1.49
2.80	1.72
3.15	1.98
3.50	2.20

[a] 25.0°, pH 6.4 phosphate buffer, μ = 0.3.

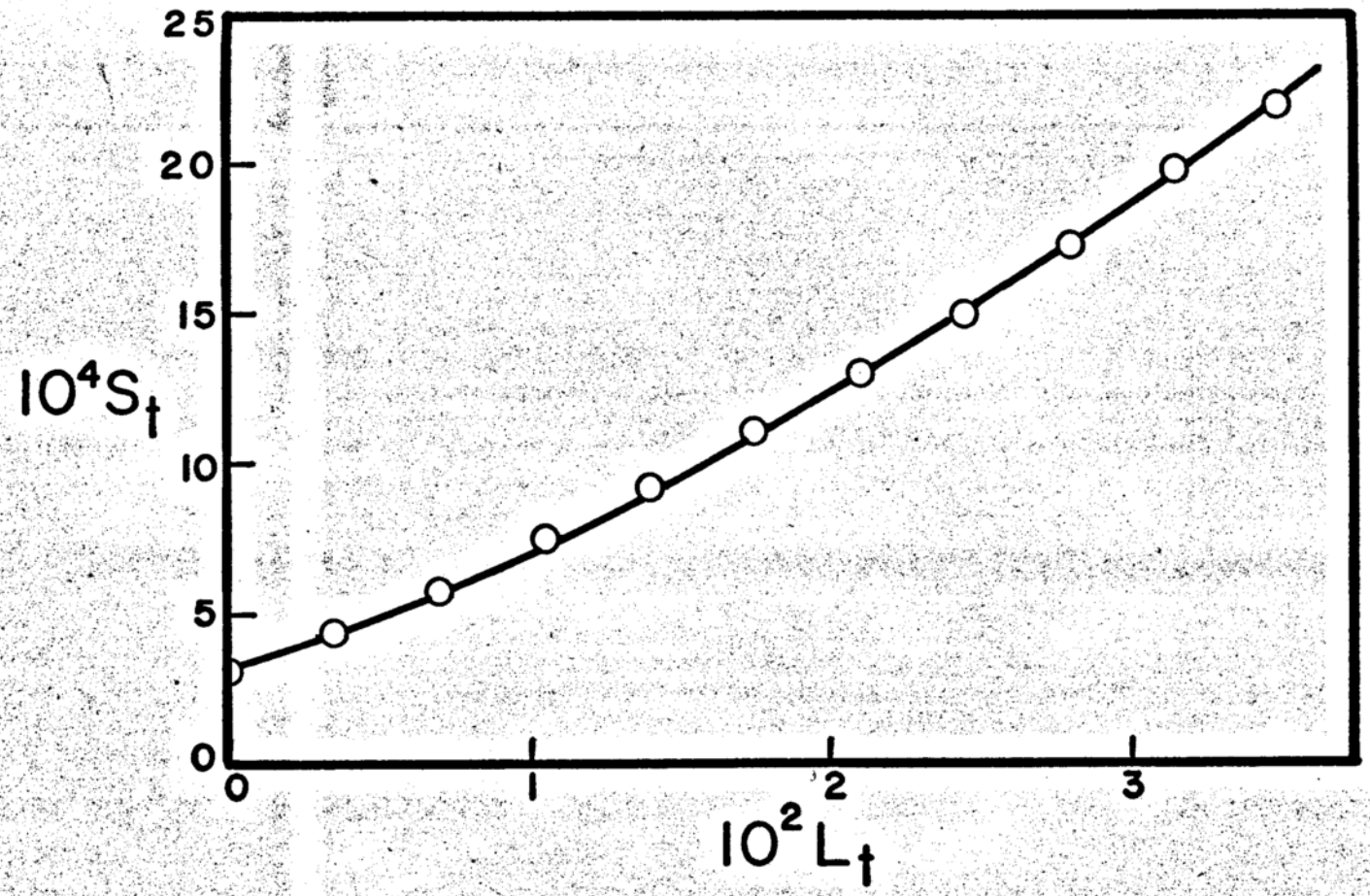


Figure 22. Plot of apparent solubility of methyl 1-naphthoate as a function of theophylline concentration. (See Table XXIII for conditions.)

TABLE XXIV

Apparent Solubility of Methyl 2-Naphthoate at Various Theophylline Concentrations[a]

Theophylline (10^2M)	Methyl 2-Naphthoate (10^4M)
0.00	0.809, 0.835
0.175	1.01
0.350	1.18
0.525	1.37
1.05	2.00
1.40	2.50
1.75	3.02
2.10	3.56
2.45	4.23
2.80	4.82
3.15	5.46
3.50	6.10

[a] 25.0°, pH 6.4 phosphate buffer, μ = 0.3.

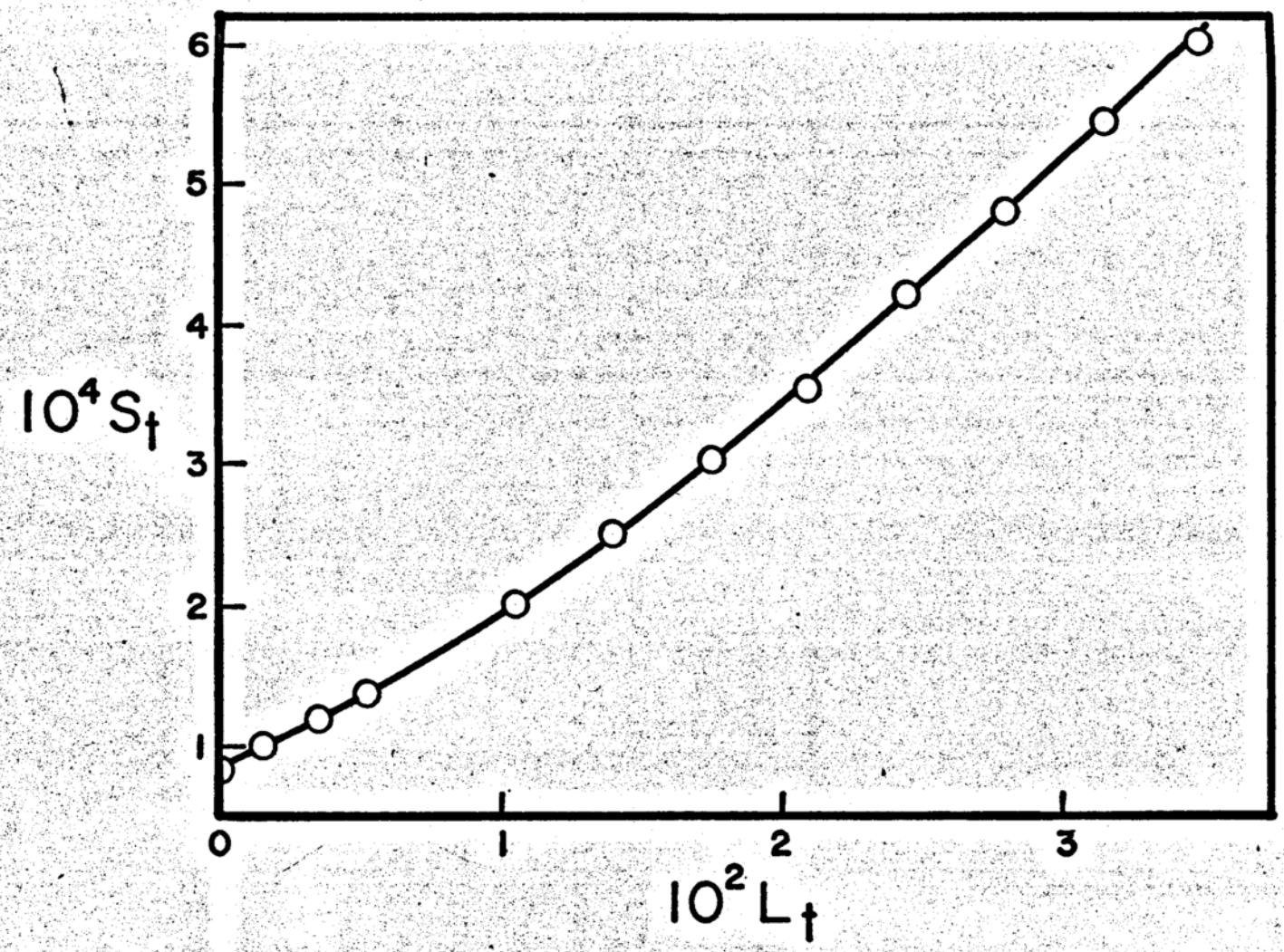


Figure 23. Plot of apparent solubility of methyl 2-naphthoate as a function of theophylline concentration. (See Table XXIV for conditions.)

as $(S_t - S_o)/L_t$ *vs.* L_t, which should give a straight line if the system does indeed contain only SL and SL_2 complexes. From the slopes and intercepts of these plots may be estimated the values of K_{11}' and $K_{(12)}$, as given by the following equations.

$$K_{11}' = \text{Y-intercept}/S_o$$

$$K_{(12)} = \text{slope/Y-intercept}$$

Figures 24 and 25 illustrate this treatment of the solubility data for the naphthoate esters in the presence of theophylline. Both plots are described reasonably well by straight lines. Although experimental data were obtained at ligand concentrations below 0.01 M theophylline, these data were not used in Figures 24 and 25. It was found that the calculation of $(S_t - S_o)/L_t$ in this region was subject to much error, even from relatively precise data. For example, a point which deviated 1.8% from the smooth curve in Figure 22 at 0.007 M ligand concentration brought about a 6% deviation in the value of $(S_t - S_o)/L_t$. Because of the expanded nature of the Y-axis in Figures 24 and 25, a 6% fluctuation in the value of $(S_t - S_o)/L_t$ is quite apparent and misleading. The stability constants evaluated from Figure 24 for the methyl 1-naphthoate-theophylline system are $K_{11}' = 104\ M^{-1}$ and $K_{(12)} = 16\ M^{-1}$. The constants computed from Figure 25 for the methyl

Figure 24. Graphical estimation of K_{12}' and $K_{(12)}$ from a solubility study on the methyl-methylphosphonic-ethanol system.

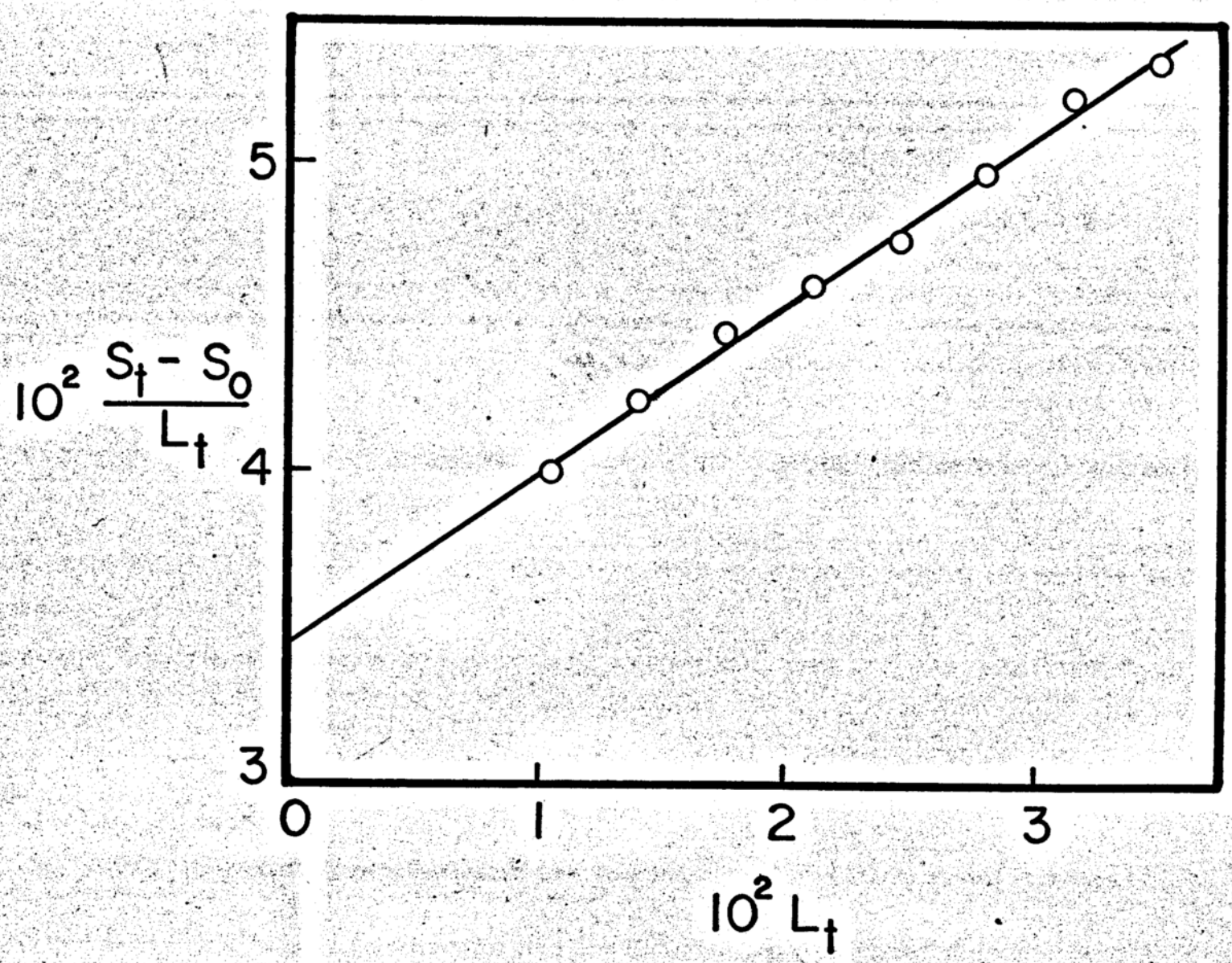
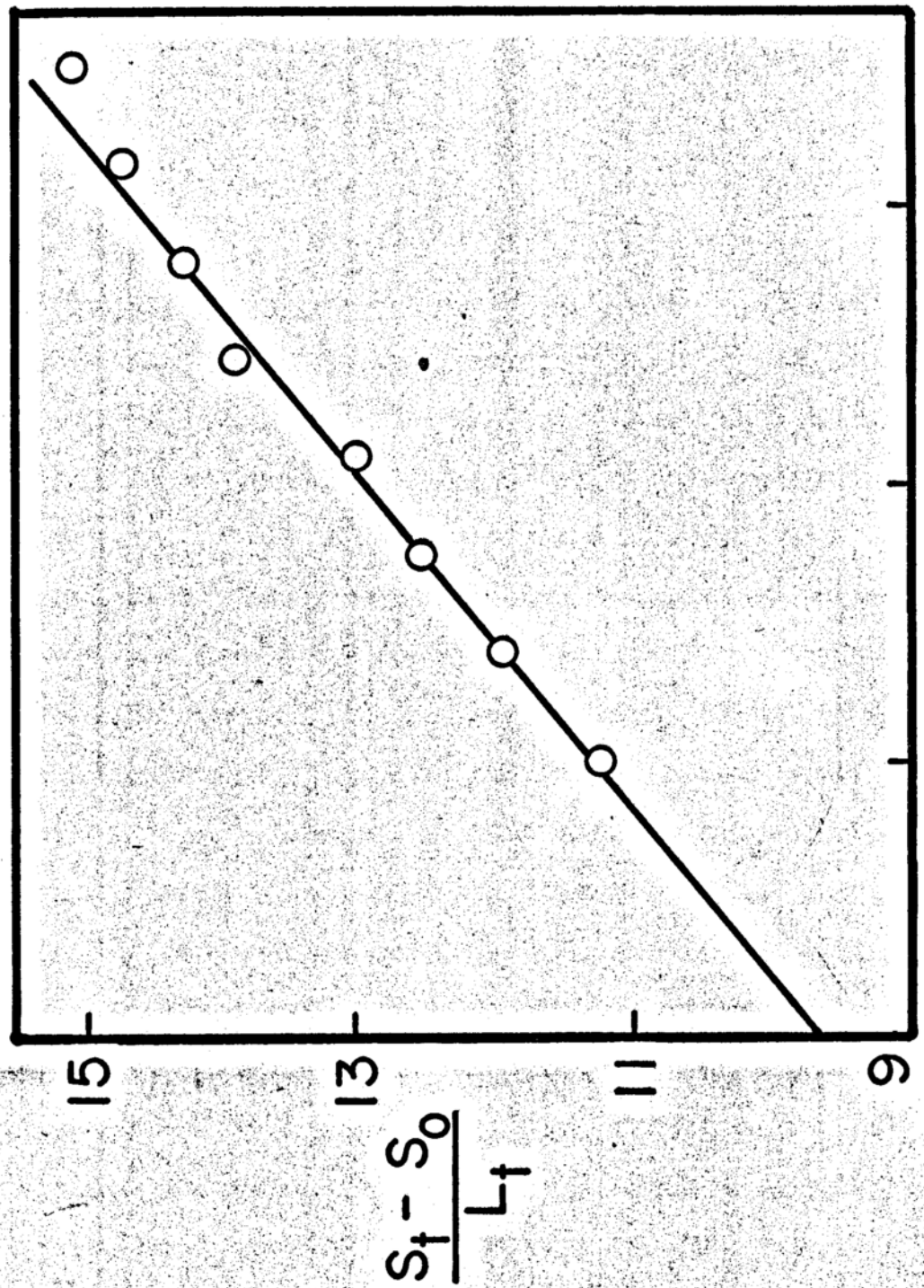

$10^2 \frac{S_t - S_0}{L_t}$
5
4
3
0
1
2
3
$10^2 L_t$

Figure 29. Graphical estimation of $K^{JJ'}$ and $K^{(JS)}$ from a solubility study on the methyl 2-naphthoate-tropylium system.


15
13
11
9
0
1
2
3
$10^2 L_t$
$\frac{S_t - S_0}{L_t}$

2-naphthoate-theophylline interaction are $K_{11}' = 116\ M^{-1}$ and $K_{(12)} = 17\ M^{-1}$. Some implications of these values will be discussed later. Because of the complications introduced by the apparent presence of the higher order complexes, kinetic and spectral studies were not attempted with these systems.

I. Methyl Hydrocinnamate.--This compound ($C_6H_5CH_2CH_2COOCH_3$), represents an interesting modification in the basic cinnamate structure. It was hoped that data obtained from studies on this ester would provide information on the importance of the double bond in the complexing of cinnamates. Methyl hydrocinnamate exhibited second-order kinetics under conditions of alkaline hydrolysis at 25.0°. Imidazole appeared to inhibit the rate of hydrolysis; therefore kinetic studies involving imidazole and acetonitrile as additives were carried out. Table XXV is a compilation of the results from these experiments. At an additive concentration of 0.50 M, acetonitrile apparently inhibits the hydrolysis rate to a greater extent than does imidazole. At the lower additive concentrations, the magnitude of the inhibitions caused by the two compounds is about the same. Since we have assumed in previous studies that acetonitrile is exerting its inhibitory action by a non-specific medium (activity coefficient) effect, the tentative conclusion drawn from these results is that imidazole, if it interacts with methyl hydrocinnamate at

TABLE XXV

Apparent Second-Order Rate Constants for the Alkaline Hydrolysis of Methyl Hydrocinnamate in the Presence of Imidazole and Acetonitrile

Additive (M)	k_s' ($M^{-1}sec^{-1}$)[a]
0.00	0.179
Imidazole	
0.10	0.168, 0.175
0.35	0.154, 0.159
0.50	0.146, 0.149
Acetonitrile	
0.10	0.164, 0.172
0.35	0.161
0.50	0.130, 0.136

[a] 25.0°, pH 12.4-12.5, μ = 0.3, based on activity measurements.

all, does so very weakly, and the inhibitory effects brought about by such interaction cannot be distinguished from solvent effects.

Kinetic studies on methyl hydrocinnamate in the presence of theophyllinate proved to be difficult experimentally and were temporarily abandoned in favor of solubility studies with theophylline as the ligand. However, spectral anomalies were encountered with this ester that were not as subtle as those which we observed for methyl 2,6-dichlorocinnamate and methyl p-nitrocinnamate. In Figure 26, the spectrum labelled A is that of methyl hydrocinnamate in aqueous buffer and at a concentration below that of saturation. Spectrum B is that of an aqueous buffer solution saturated with respect to methyl hydrocinnamate. Our first assumption was that the ester was undergoing self-association at saturation concentrations; however, dilution of saturated solutions failed to change the shape of the spectra from that of spectrum B. Numerous other experiments were conducted on these systems to determine if hydrolysis, light, temperature, oxygen, Parafilm, and other factors were responsible for the spectral anomalies. None of these, however, appeared to be the cause of this spectral change. Spectrum A was reproducible to the extent that several Beer's Law plots in buffer were carried out in which this spectrum was the one measured at various concentrations.

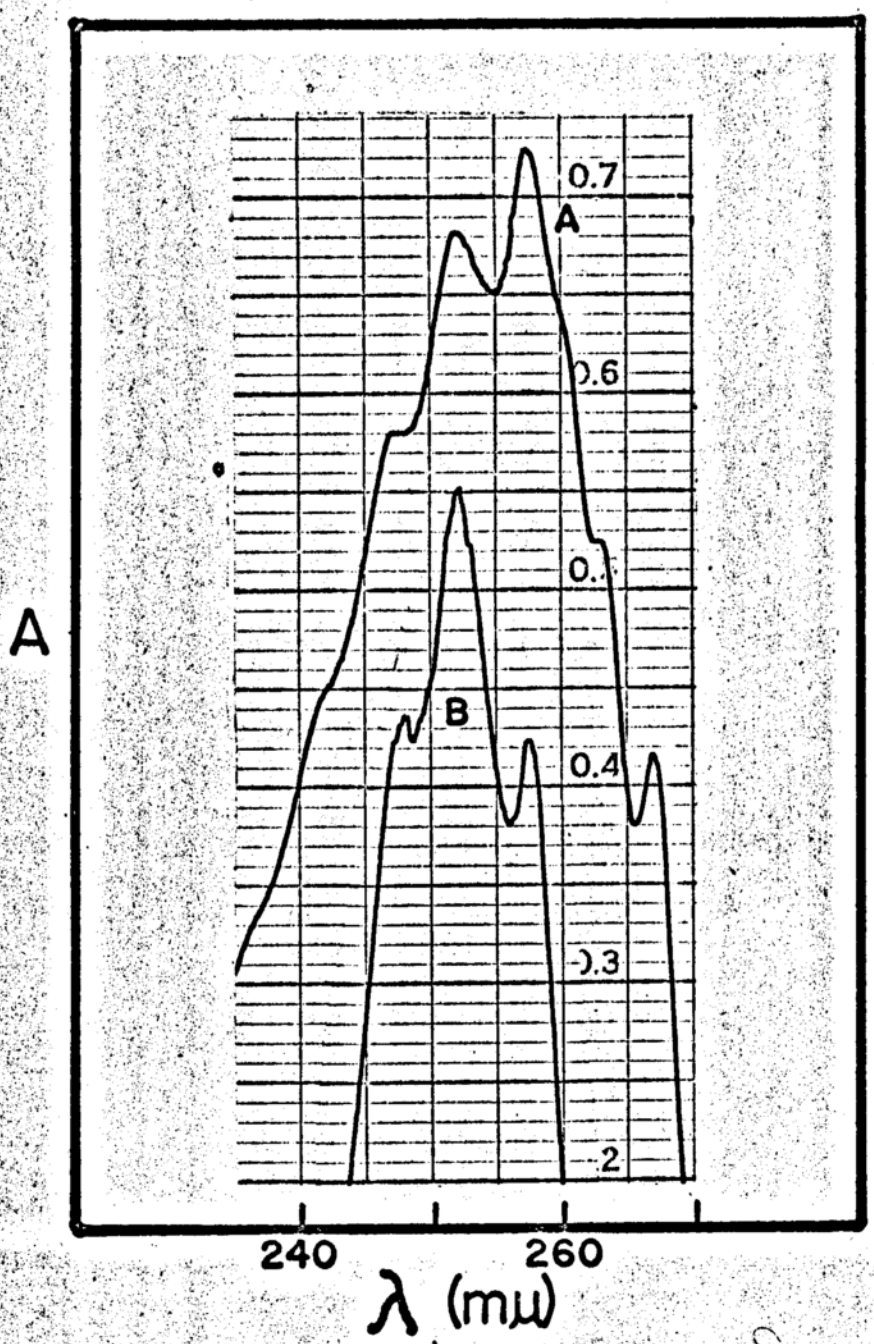
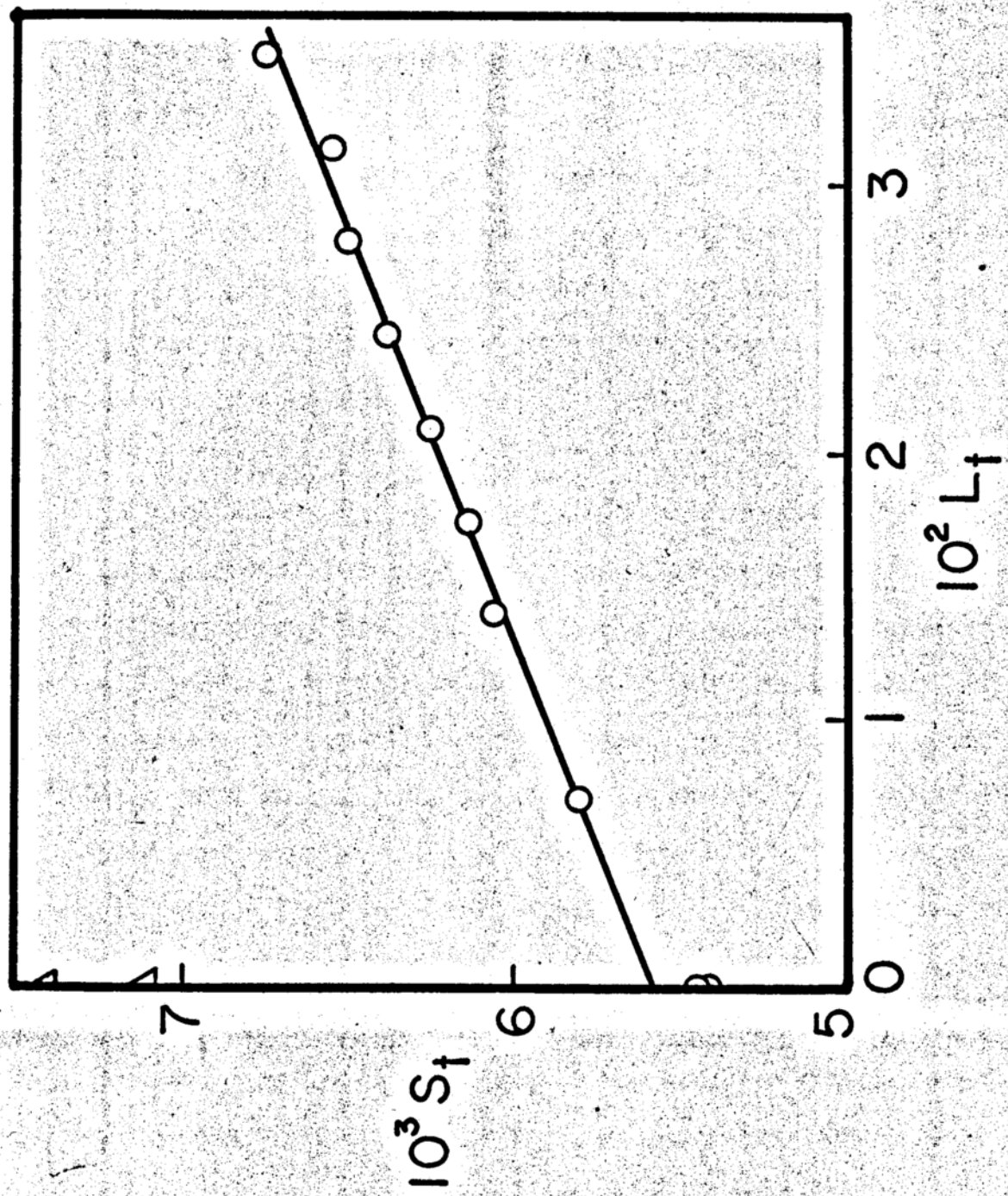


Figure 26. Plot of absorbance vs. wavelength for methyl hydrocinnamate in aqueous buffer at 25.0°. (Spectra shifted vertically for clarity).

The molar absorptivity calculated from these plots was satisfactorily reproducible. We never were able to convert spectrum B into spectrum A, however. It was an easy task to convert A into B, for all that was required was to place a solution having spectrum A in contact with excess ester for a few minutes; a scan of the supernatant liquid always produced spectrum B. One final point to be made is that only one spectrum could be obtained for methyl hydrocinnamate in isooctane. When aqueous solutions having spectra A or B were extracted with isooctane the same spectrum resulted in either case, and it was identical with that obtained from solutions prepared by dissolving methyl hydrocinnamate in isooctane.

In spite of the spectral anomalies, curiosity led to a solubility study being made on the methyl hydrocinnamate-theophylline system, the analysis being performed by isooctane extraction of the ester. The results of this study are presented in Figure 27. The increase in the apparent solubility of methyl hydrocinnamate appears to be linear with increasing theophylline concentration, but the line drawn through the points has no clear significance because of the disagreement between the Y-intercept and the experimentally determined values of S_o. The triangles on the Y-axis represent values of S_o determined by measuring spectrophotometrically at 258 mμ the aqueous phase before extraction with isooctane. The apparent stability constant calculated from the line in Figure 27

Figure 27. Plot of apparent solubility of methyl
hydrocinnamate in the presence of various
thiophylline concentrations:
○- determined by extraction with
isooctane; △- see results.


$10^3 S_t$
7
6
5
0
1
2
3
$10^2 L_t$

is 6 M^{-1}, but its meaning is certainly questionable. The spectral anomalies also cast doubt on the validity of the kinetic studies with imidazole and acetonitrile. Although the kinetic measurements were consistently made on solutions having spectrum A, the significance of such measurements is questionable since the identity (or identities) of the species giving rise to spectrum A is not known. A possible explanation for the origin of these spectral differences will be offered in the discussion section of this dissertation.

J. Styrene.--Styrene, (C_6H_5-CH=CH_2), is a logical member in this series of substrates, since cinnamates are derivatives of styrene. We had hoped that the results of complexing studies with styrene would provide insight into the importance of the ester functional group in the interaction of cinnamates with xanthine ligands. Unfortunately styrene was experimentally very difficult to study under the conditions used in our systems. Solubility studies in the presence of theophylline were attempted, but the data were quite scattered when plotted on a solubility isotherm. This is not surprising in view of the many possible modes of styrene decomposition referred to in reference (37). The volatility of styrene also presented many difficulties. Steps were taken to reduce the problems brought about by volatility, and nitrogen was used in the headspace of the

solubility vials with the hope of reducing the decomposition rates. These measures had no noticeable effects, however, on the success of the solubility studies. Theophylline appeared to increase the solubility of styrene in aqueous buffer as indicated by the trend in the solubility isotherms. The magnitude of this increase suggested a high degree of interaction with theophylline, but no effort was made to calculate stability constants from the data because of its poor reproducibility. Spectral studies were attempted on freshly prepared solutions of styrene in the presence of theophylline. However, theophylline apparently did not shift the spectrum of styrene at analytically useful wavelengths, and therefore it was not possible to evaluate a stability constant by the spectral technique.

IV. DISCUSSION

A. Complexation Dependence on Acyl Group Structure.--The following discussion will be based on the results of the interactions between the various substrates with theophylline relative to the interaction of methyl trans-cinnamate with theophylline. Table XXVI is a compilation of these results.

The results of the methyl cis-cinnamate-theophylline system support the proposal that the xanthine-substrate interaction is favored by a parallel orientation of molecular planes. Molecular models indicate that methyl trans-cinnamate can assume a planar configuration. These models also show that methyl cis-cinnamate cannot exist in a planar configuration; the side chain double bond and carbonyl can be coplanar with each other but not with the aromatic ring. This configuration is supported by the recent nmr study of Wittstruck and Trachtenberg (51). Table XXVI indicates that approximately a 50% reduction in the degree of interaction between cinnamates and theophylline occurs when the configuration is changed from trans to cis. This decrease can be rationalized on the basis of steric effects and/or electronic properties. For example, one possibility is that the loss of planarity in the cinnamate structure and the resulting decrease in conjugation between the aromatic ring and side chain may

TABLE XXVI

Apparent Stability Constants with Theophylline[a]

Substrate	Theophyllinate Kinetic	Theophylline Solubility	Theophylline Spectral
$C_6H_5CH=CH-COOCH_3$ (trans)	11[b]	25[c]	22[c]
$C_6H_5CH=CH-COOCH_3$ (cis)	7	11	16[d]
$C_6H_5COOCH_3$	16	[e]	[f]
$CH_3-CH=CH-COOCH_3$ (trans)	5	2.1	[f]
$C_6H_5CH_2CH_2COOCH_3$	---	5.9	[f]
CH_3COOCH_3	0	---	---
$C_6H_5CH=CH-CONH_2$ (trans)	17[g] 10[h]	28	17
$C_6H_5CONH_2$	---	15	---
Methyl 1-Naphthoate	---	104[i]	---
Methyl 2-Naphthoate	---	116[j]	---
Methyl 2,6-Dichloro-trans-cinnamate	---	39	[f]
Methyl p-Nitro-trans-cinnamate	---	21	[k]
$C_6H_5CH=CH_2$	---	[e]	[f]

[a] Expressed as K_{11}' in M^{-1}.

[b] Data from reference (1).

[c] Unpublished data from Mr. J. L. Cohen.

[d] Evaluated at two different wavelengths.

[e] Definite solubility increase, but no K_{11}' evaluated; see Results.

[f] No spectral shift at analytical wavelengths.

[g] Solubility method.

[h] Spectral method.

[i] $K_{(12)} = 16\ M^{-1}$.

[j] $K_{(12)} = 17\ M^{-1}$.

[k] See Results.

lead to a reduction in the ability of the ring to act as an electron acceptor (assuming this to be a requirement for interaction). A second possibility for the reduced interaction with the cis isomer arises if we assume that the coplanar double bond and carboxyl function are primarily responsible for the cinnamate-xanthine interaction. In the cis isomer the carboxyl group and double bond can be coplanar. The reduced interaction could then result from steric hindrance by the ring to the approach of the xanthine molecule. A third possibility is that the combined steric and electronic properties of the planar trans isomer are necessary for optimum interaction. When these are altered, as in the cis compound, reduced interaction results.

In the recent literature have appeared many studies which indicate a preference for parallel plane orientation between the interactants of a molecular complex (52-55). Most of these interactions have been postulated to result from charge-transfer forces. If charge-transfer is responsible for these interactions, it is not difficult to understand the reason for the parallel-plane orientation, since maximum orbital overlap is achieved by this configuration. Much recent literature on charge-transfer complexes indicates that electron transfer may be important in the excited state, but that in the ground state, van der Waal's forces appear to be primarily responsible for the interaction. In 1961, Wallwork concluded that polarization

bonding* was more important than electron transfer in interactions between aromatic molecules in the crystal lattice (56). Le Fevre, et al., measured dipole moments of hexafluorobenzene in benzene and mesitylene, but found no spectroscopic evidence for charge-transfer interactions (58). They conclude that stabilization of the ground state of donor-acceptor complexes is determined to a greater extent by van der Waal's interactions than by charge-transfer. Hanna (59) and Matsuo and Higuchi (60) also propose that van der Waal's forces are important in donor-acceptor complexes in the ground state. It is interesting to note that maximum orbital overlap is not required for dipole interactions to occur. The only requirement is that the dipoles (or induced dipoles) be aligned in an electrostatically favorable orientation. In proposing a structure for the methyl trans-cinnamate-theophylline complex, it would help if we knew which type of forces are largely responsible for the interaction because of the different geometrical requirements of charge-transfer and dipole interaction. That a spectral shift occurs when some cinnamate esters interact with theophylline is not evidence for charge-transfer interaction alone since spectral shifts obviously can be induced by other types of interactions. With the hope of resolving some of these uncertainties, let us examine several of the other results in Table XXVI.

*Polarization bonding is a generic term coined by McKeown, Ubbelohde and Woodward (57) to describe induced-dipole as well as charge-transfer interactions.

In discussing the results of the methyl cis-cinnamate-theophylline interaction, we offered the possibility that the coplanar, contiguous double bond and carboxyl group may be responsible for the complexation and that the ring may have been sterically blocking the approach of the xanthine molecule. The results of the interaction between methyl crotonate and theophylline should determine if this is plausible, since this molecule possesses the coplanar, contiguous double bond and carboxyl group, and the methyl group on the acyl portion of the ester should offer little steric hindrance to the approach of the xanthine molecule. Methyl crotonate does not interact nearly to the extent that methyl trans- or cis-cinnamate interact. This result strongly implicates the aromatic ring in the interaction process. Other results in Table XXVI which also emphasize the participation of the aromatic ring are those with methyl benzoate, cinnamamide, benzamide, and the naphthoates. The literature certainly supports the importance of aromatic rings in molecular complexes. Nmr data suggest that N,N-dimethylformamide interacts with aromatic solvents (61). The anhydride, esters, and amide of 3,5-dinitrobenzoic acid all form colored solid complexes with polycyclic aromatic donors (62). Of historical and theoretical significance are the many benzene-halogen complexes (10). A large tabulation of the interactions between many aromatic compounds and other aromatic and heterocyclic molecules appears in reference (45).

The importance of conjugation between the ring and some other group containing π-electrons is illustrated by comparing the results for methyl benzoate, benzamide, methyl hydrocinnamate and methyl acetate. The contribution of the carboxylate group to the interaction is shown by the low degree of interaction (if any) between theophyllinate and methyl acetate. Placing an aromatic ring in the same molecule with a carboxyl group apparently increases the degree of interaction, as seen by the result with methyl hydrocinnamate, but not nearly to the extent as with methyl benzoate and benzamide, where the ring and carboxyl group are conjugated.

It is not difficult to rationalize (though it is not so easy to predict) the higher stoichiometry (SL_2) in the interaction of the naphthoates with theophylline. The increased planar surface created by the addition of another aromatic ring to the substrate acyl group is probably responsible for the additional interaction. What is most difficult to explain, however, is the large increase in the degree of interaction as indicated by the large K_{11}' values.

The results of the interaction between methyl 2,6-dichlorocinnamate and theophylline raise some interesting questions. Models and X-ray data (63) indicate that the molecule cannot be planar and, hence, the resonance interaction between the aromatic ring and the side chain is reduced. On this basis we would not expect this

ester to interact with theophylline to the same extent as did methyl trans-cinnamate. However, we can see that our prediction does not bear up under the experimental results. We have neglected several other important contributions of the chlorine atoms to the ester molecule. If the aromatic ring were acting as an electron acceptor, the chlorine atoms would tend to increase the degree of interaction in such a situation because they can, in effect, withdraw electrons from the ring. If the interaction with theophylline were a dipole-dipole interaction, the chlorine atoms might intensify the dipole of the ester molecule and increase the extent of interaction. A third possibility is that of direct interactions between the chlorine atoms and the theophylline molecule. This interaction may be of a donor-acceptor nature similar to that between acetone and bromine molecules, where the oxygen atom of acetone serves as a non-bonded electron donor for the acceptor bromine molecules (64). Robinson and Jencks observed that the presence of chloride and iodide substituents on anions augmented "salting-in" effects which they later ascribed to a specific complexation interaction (65).

Several times in the course of this discussion we have suggested the possibility that the aromatic ring in the cinnamate esters may be acting as an electron acceptor. If this were true, then methyl p-nitrocinnamate should interact quite strongly with theophylline because the electron-withdrawing nitro group should enhance the

electron-accepting properties of the ring. The results in Table XXVI do not support this, however. It appears that methyl p-nitrocinnamate interacts with theophylline to about the same extent as does methyl trans-cinnamate. There is a plausible explanation for this result based on dipole-dipole interactions. Methyl trans-cinnamate is a polar molecule with its negative pole directed toward the carboxylate portion of the molecule and its positive pole in the vicinity of the aromatic ring. Theophylline is also polar, and it would interact with methyl trans-cinnamate in a typical dipole-dipole manner with the dipoles opposing each other. Now place a nitro group para to the side chain of methyl trans-cinnamate. The dipole moment of methyl trans-cinnamate is 1.95 D (66). The group moment of an aromatic nitro group is 4.01 D (67). In methyl p-nitrocinnamate these moments are nearly directly opposed, so the vector difference, or 2.06 D, may be taken as an estimate of the net dipole moment of methyl p-nitrocinnamate. This happens to be a poor estimate, for the measured moment of methyl p-nitrocinnamate is 3.53 D (66); however, the essential point is that the sense of the moment is reversed in the p-nitro ester (relative to the unsubstituted ester). This dipole reversal probably changes the geometry of the complex with theophylline, so that a comparison of stability constants would no longer be valid.

At no time in this discussion have we made mention of hydrogen bonding as a possible influence in these interactions. Although we cannot rule out hydrogen bonding as a contributing factor, we do not feel that it is of major importance in these interactions. The structural variations made during this study were primarily on the acyl portion of ester molecules. The carbonyl oxygen of the ester would be the atom most likely to participate in hydrogen bonding; it is unlikely that some of these variations could bring about such drastic changes in the hydrogen-bonding acceptor properties of the carbonyl oxygen so as to cause the observed changes in K_{11}'.

Hydrophobic bonding is certainly a possibility in these interactions. It may be that hydrophobic bonding is the major force holding together the complex while the dipole-dipole interactions merely determine the general orientation of its components. It is interesting to note that liquids exert a mechanical pressure of 10^3-10^4 atmospheres on dissolved molecules (68). Is it not possible that such forces could aid in the interaction of two favorably oriented dipoles?

B. Related Studies.--(1) 8-Bromotheophyllinate.--No useful conclusions can be drawn from the results of the interactions between several substrates and 8-bromotheophyllinate; see Table XXVII. Agreement between the solubility

TABLE XXVII

Apparent Stability Constants with Imidazole and 8-Bromotheophyllinate[a]

Substrate	Imidazole Kinetic	8-Bromotheophyllinate Solubility	8-Bromotheophyllinate Spectral	8-Bromotheophyllinate Kinetic
C_6H_5-CH=CH-$COOCH_3$ (trans)	0.9	30	28	--
C_6H_5-CH=CH-$COOCH_3$ (cis)	0.07	13	24 27[b]	7.5
$C_6H_5COOCH_3$	0.9	[c]	23 13[b]	[d]
CH_3CH=CH-$COOCH_3$ (trans)	0.5	--	--	--
$C_6H_5CH_2CH_2COOCH_3$	~0[e]	--	--	--
CH_3COOCH_3	~0[f]	--	--	--

[a] Expressed as K_{11}' in M^{-1}.

[b] Determined at two different wavelengths.

[c] Definite solubility increase, but no K_{11}' evaluated; see Results.

[d] Kinetic reciprocal plot curved.

[e] See Results.

[f] Inhibitions of same magnitude as with comparable CH_3CN concentrations.

and spectral stability constants suggests that this ligand may be interacting in a 1:1 stoichiometry with methyl trans-cinnamate; however, the data for methyl cis-cinnamate and methyl benzoate most certainly signify the presence of multiple complexes. Although we did not predict this behavior a priori, it is not too surprising when we consider the highly polarizable outer orbital of the bromine atom. These electrons would certainly enhance the tendency of the atom to undergo dispersion, induced dipole, and donor-acceptor interactions. Since this atom is attached to a theophyllinate molecule, it may increase the tendency of theophyllinate to undergo interactions of a higher order than 1:1.

(2) Imidazole and Acetonitrile.--The apparent stability constants for imidazole and various substrates are presented in Table XXVII. The significance of these results is not easily ascertained. Throughout the Results section of this dissertation we have compared percent of rate inhibitions for identical concentrations of acetonitrile and imidazole. The assumption was that acetonitrile was acting only through a non-specific medium (solvent) effect while imidazole, in addition to the inevitable solvent effects, was undergoing a specific complexation interaction with the ester, thereby reducing its susceptibility to hydrolysis. The results of comparative studies on methyl acetate appeared to lend support to this assumption since imidazole and acetonitrile influenced the hydrolysis rate of this

ester to the same extent at comparable concentrations. Attempts at finding literature support for this assumption, however, met with questionable success. For example, solvent effects can sometimes be accounted for by application of the Setschenow equation (69). This equation predicts that a plot of log γ vs. concentration of added substance will be linear if solvent effects alone are responsible for changes in the measured property of a dissolved solute. We could not apply the Setschenow equation directly to our data because we had not measured activity coefficients in our systems. Menger and Portnoy have applied the Setschenow equation to rate data for an ester hydrolysis by plotting log of the rate constant vs. additive concentration (70). Although they do not elaborate on this treatment, such an application is valid only if the ratio $(\gamma_{OH^-})(\gamma_E)/\gamma_{X^*}$ is constant, where the γ's are activity coefficients and the subscripts OH^-, E, and X* signify hydroxide ion, ester, and transition state, respectively. This ratio apparently was constant for their system because they claim that the plot was linear. A plot of log k_{OH} vs. additive concentration for our data on methyl acetate with imidazole and acetonitrile was not linear; this signifies either that solvent effects alone are not responsible for the observed rate decreases, or that the ratio γ_E/γ_{X^*} is not constant for our system. The latter possibility is given some support by

the work of Villermaux, et al., who attempted to correlate activity coefficients and second-order rate constants for the alkaline hydrolysis of a homologous acetate ester series (71). Methyl acetate did not obey the same relationship as did the higher members of the series. These workers conclude that this may be because the ratio $\gamma_{OH^-}/\gamma_{X^*}$ is not constant throughout the series. That our data for methyl acetate does not obey the Setschenow equation cannot be taken, therefore, as conclusive evidence that some effect other than solvent effects are operative in this system.

In an effort to find support for our assumption that only solvent effects were responsible for the observed inhibitions of methyl acetate hydrolysis, we turned to the literature for data on the dielectric constant of dilute aqueous imidazole and acetonitrile solutions. Devoto measured the dielectric constant of aqueous acetonitrile solutions and found $d\epsilon/dc$ to be -1.74, where ϵ is the dielectric constant and c is the molar concentration (72). Hückel, et al. did the same for imidazole solutions and found $d\epsilon/dc$ to be zero (73). On the basis of these data, one might conclude that imidazole and acetonitrile cannot exert their influence by the same mechanism. However, Kosower warns that extreme caution must be used in interpreting macroscopic dielectric constants as "a reference parameter on the microscopic level" (74). The methyl acetate system may be an example of a system in

which this warning should be heeded. In aqueous solution, methyl acetate is solvated by the hydrolytic species. It appears from the dielectric constant measurements that imidazole fits rather easily into the structure of water. Is it not feasible that several molecules of imidazole will be included in the solvent sheath surrounding a methyl acetate molecule? Thus imidazole could interfere with the movement of the catalytic species through the solvent sheath, since imidazole could not participate in the transfer of hydroxide ion through water in the manner proposed by Frank and Wen (75). Another possibility is that incorporation of imidazole into the solvent sheath reduces the activity of water in the vicinity of the ester molecule. However, a plot of mole fraction of water *vs.* imidazole concentration for this system is linear up to 0.5 M. Since the plot of k_{OH} *vs.* additive concentration is hyperbolic, we feel that reduction of water activity is not solely responsible for the observed retardation. The result of these rationalizations is that we still have no firm evidence for or against the solvent effect hypothesis.

It might be possible to support the solvent effect hypothesis by demonstrating the absence of a complexation interaction. In attempting to do this, we plotted the methyl acetate-imidazole data on a $k_s/\Delta k_s$ *vs.* $1/L_t$ plot. The trend (except for one point) was reasonably linear, and a K_{11}' of 0.6 M^{-1} was calculated. Here we have

evidence that some sort of specific interaction between methyl acetate and imidazole may be occurring. We are also reminded of Allen's work in which 0.1 N sodium glycollate caused 6.7% retardation in the rate of methyl acetate saponification while 0.1 N sodium salicylate retarded the rate by 33.4% (7). This may be evidence for a specific interaction between methyl acetate and sodium salicylate. On the other hand, Blackburn and Jencks, in a recent study, showed that the rate of methyl formate aminolysis by morpholine is retarded 12% by 0.5 M dioxane* (76); they attribute this to a solvent effect. In our system, the rate of methyl acetate hydrolysis is retarded 17% by 0.5 M imidazole or acetonitrile. It is difficult to determine, on the basis of these relative rate effects, the dividing line between solvent effects and specific interaction.

In view of these many contradictory results we make the following conclusions:

1) Methyl acetate cannot be used as a model compound for discriminating between solvent effects and complexation due to imidazole and acetonitrile.

2) The complexation hypothesis for esters such as methyl trans-cinnamate and methyl benzoate with imidazole may still be valid, but if it were possible to subtract

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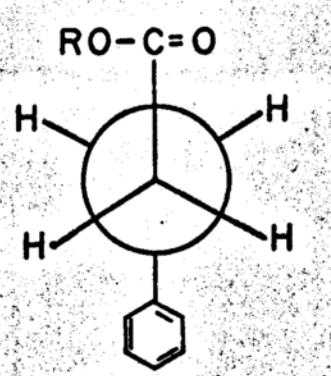
*Estimated from a graph; data not given.

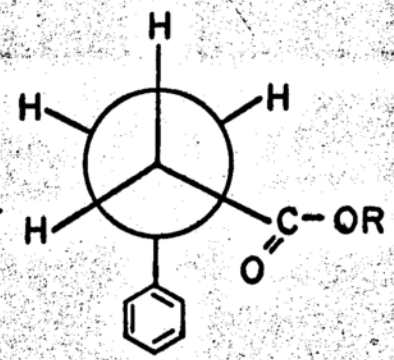
solvent effects from the observed inhibitions, the apparent stability constants might be of very small magnitude. For a detailed discussion on the validity of complexation _vs_. solvent effects based on very small differences in measured properties, see reference (77).

3) Because of the uncertainties in these systems, no attempt will be made at this time to postulate structures for a methyl _trans_-cinnamate-imidazole complex.

(3) Methyl Hydrocinnamate.--This discussion will bear on a possible explanation for the two different UV spectra exhibited by methyl hydrocinnamate in aqueous solution. Methyl hydrocinnamate is a disubstituted ethane; its conformations can be illustrated by the familiar Newman projections, below.



trans



skew

A third possible conformation (eclipsed) has been omitted because the probability of the molecule existing in that rotational form is extremely low. Rotation about the carbon-carbon single bond of the ethane molecule is now somewhat restricted due to the non-bonded interactions between the phenyl ring and the ester group. Thus there is an equilibrium between the two conformations, the position of the equilibrium being determined by the free energy difference between them. For example, 1,2-dichloroethane prefers the trans conformation at room temperature, but as the temperature is increased, the equilibrium is shifted to favor the skew conformation as shown by dipole moment measurements (78).

Molecular models indicated that methyl hydrocinnamate could assume a configuration which placed the carbonyl oxygen near the aromatic ring in such a manner that one could envision an interaction between these two functional groups. Interestingly, support for this proposal was found in the literature. In their nmr study, Wittstruck and Trachtenberg propose just such an interaction (they call it an intramolecular acid-base interaction) to explain the unusual behavior of the chemical shifts for the α- and β-protons in hydrocinnamic acid (51). On the basis of the Newman projections, we propose that the equilibrium for methyl hydrocinnamate favors the skew conformation. The input of energy into the system in the form of light, heat, mixing, and perhaps other mechanisms unknown to us

at this time, could cause a shift in the equilibrium toward the trans conformation. It is likely that the two conformations have different UV spectra since in the skew conformation the proposed interaction involves the only two chromophores in the molecule, and in the trans conformation, little or no interaction should occur. Unfortunately, the experiments conducted in connection with the anomalous UV spectra were not performed with this hypothesis in mind. Therefore, the data obtained do not allow us to support or refute entirely the hypothesis at this time. More detailed studies using nmr techniques would probably indicate whether or not our proposal is reasonable.

C. Proposed Complex Structures.--The structures of two complexes involving caffeine and carboxylic acid derivatives have been discussed in the recent literature (79,55). The caffeine-benzocaine complex structure has not been determined experimentally, but was proposed by Schnaare and Martin on the basis of electron density calculations for each molecule (79). The crystal structure of the complex between caffeine and 5-chlorosalicylic acid has been determined by the X-ray diffraction method (55); Shefter admits that crystal structures may not represent the exact structure of the complex in solution, but certainly this work is valuable in indicating possible modes of interaction. (See also Wallwork's series on complex

structures in the crystalline state.) We wish to propose some possible structures for the methyl trans-cinnamate-theophylline complex in aqueous solution. This approach is somewhat intermediate to those of Schnaare, Martin, and Shefter in that it is neither a direct structure determination nor a purely theoretical treatment; instead it involves construction of models constrained by the experimental results obtained, mainly in this laboratory, from solutions of interacting components.

The proposed structures are depicted in Figure 28 (I-III); they were constructed by utilizing the following common features and assumptions:

1) The molecular planes of the interactants are assumed to be parallel.

2) Some portion of the theophylline molecule is closely situated to the ester group; this is based on the observed kinetic inhibitions, which we have ascribed to complexation.

3) The aromatic ring is directly involved in the interaction.

4) The alcohol moiety of the ester does not participate directly in the interaction.*

5) The aromatic ring is not sterically or electronically disturbed to a large extent by the interaction. This is

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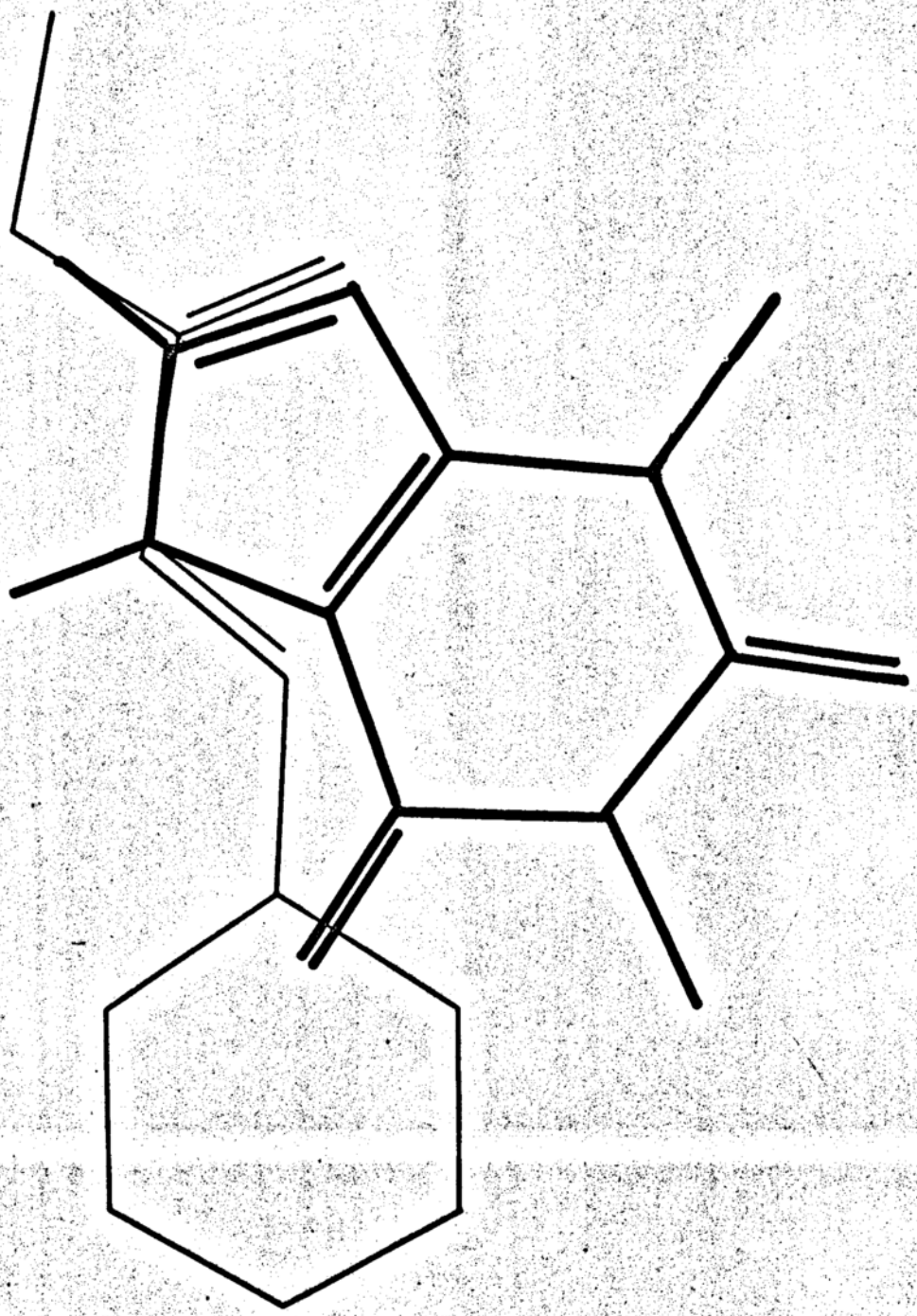
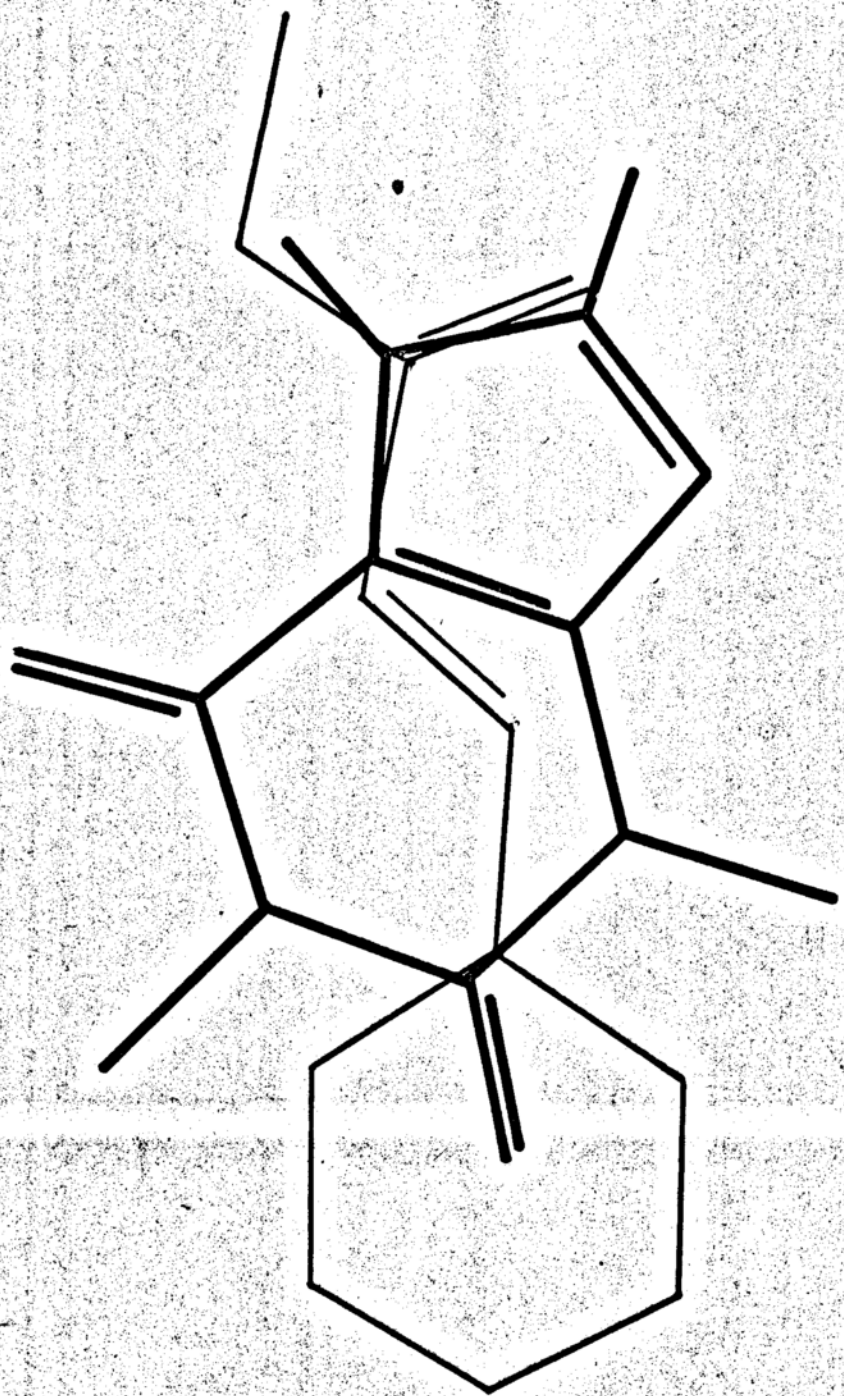
*Unpublished results of Mr. M. H. Infeld.

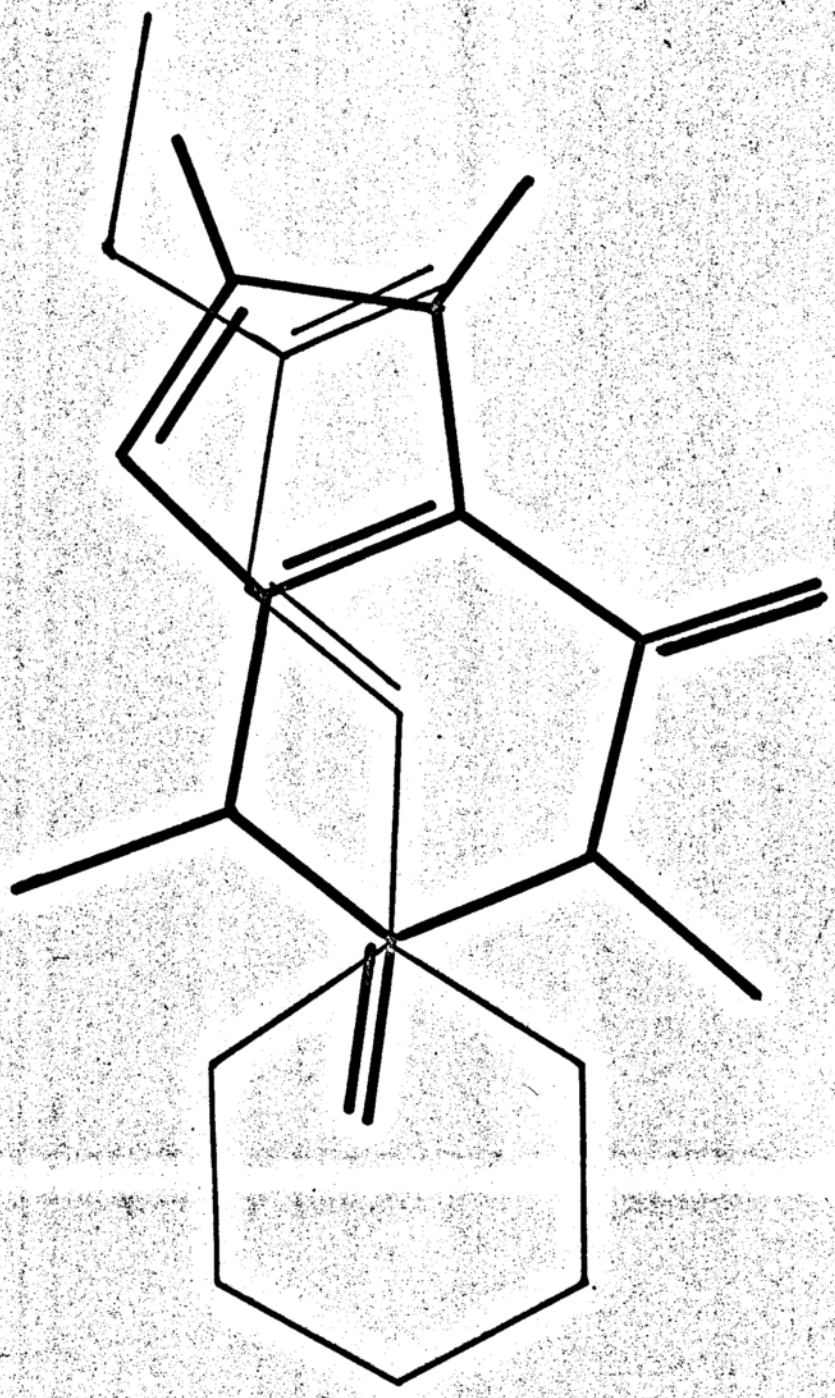
Figure 41. Base proposed mechanisms for the methyl
trans-cinnamate-copper(II) acetate (I-III) and
-triphenylphosphine (IV) complexes in aqueous
solution.



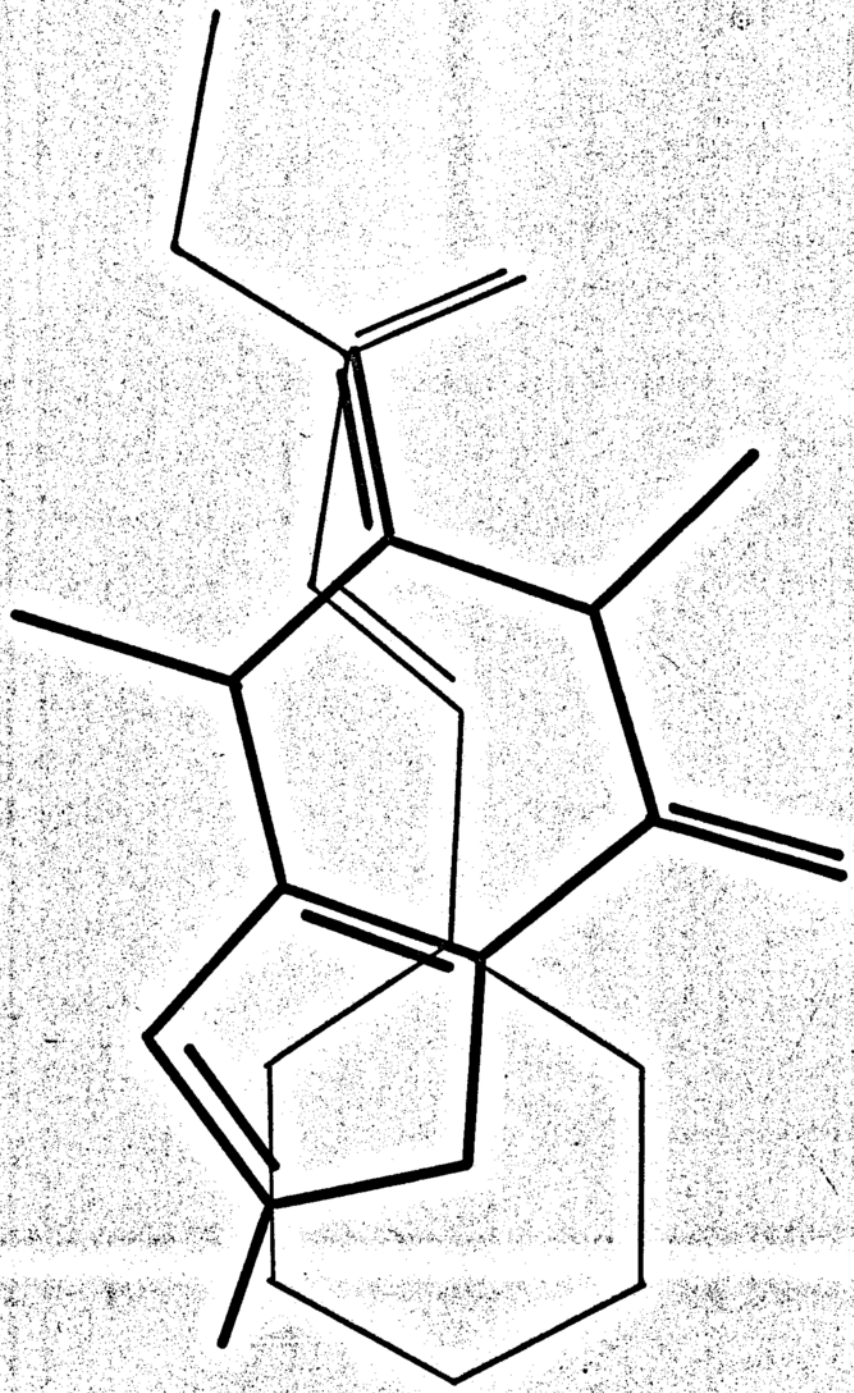
28-I



28-II



28-III



28-IV

based on the fact that acetoxy groups, substituted at various positions on the aromatic ring of trans-cinnamic acid, are not totally unreactive to hydroxide-ion catalyzed hydrolysis when the molecule is complexed with theophylline anion; i.e., $q < 1$.*

6) The general orientation of the interactants is based on dipole-dipole interactions rather than the maximum π-orbital overlap required for optimum charge-transfer interactions; however, slight changes in orientation from that for maximum dipole-dipole interaction are considered when local charge-transfer forces can confer additional stability.

7) Attempts were made to place one of the carbonyl oxygens of theophylline near the center of the substrate phenyl ring. The π electron density is lower over the center than at the perimeter of the aromatic nucleus (10) while the carbonyl oxygens of theophylline are the two most electronegative atoms in the xanthine molecule (79).

We have stated that the general orientation in our proposed structures is based primarily on favorable dipole-dipole interactions. Although dipole moment values are available for both methyl trans-cinnamate and theophylline, they do not indicate the sense of the moment. This we have

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*Unpublished results of Miss H. Stelmach.

rationalized on the basis of favorable resonance structures for the two molecules. It is reasonable to assume that the negative pole of the ester is near the carboxylate group because of its strong electronegativity. Resonance structures for theophylline indicate that a negative charge is more readily accommodated by the carbonyl oxygens of the six-membered ring, while the positive charge can be favorably localized on the five-membered ring. Based on the above reasoning, we would tend to favor structures 28-II and 28-III over 28-I because the latter may have somewhat less favorably oriented dipoles. Based on local interactions, the author tends to favor structure 28-III over the other two. For example, in structure 28-III the electronegative 2-oxygen of theophylline is situated centrally over the aromatic ring of the ester while the relatively positive 2-carbon is over the relatively negative perimeter of the ring. The interactants in structures 28-I and 28-II are not quite so favorably oriented. Notice also in structure 28-III that the relatively positive 7-nitrogen of theophylline can interact with the relatively negative carbonyl oxygen of the ester. Another possible, but admittedly minor, local interaction in structure 28-III is that between the slightly positive 4-carbon of theophylline and the negative β-carbon of the substrate ethylenic double bond. In structure 28-II, this latter weak local interaction is still possible, but one

unfavorable local interaction exists where the positive 7-nitrogen of theophylline is situated near the positive ester carbonyl carbon of the ester. There is one other weakly favorable local interaction in structure 28-II between the slightly positive 8-carbon of theophylline and the negative ester carbonyl oxygen. In addition to the possibility of unfavorably oriented dipoles, structure 28-I has two unfavorable local interactions. The positive 8-carbon of theophylline is situated near the positive ester carbonyl carbon while the negative 9-nitrogen of theophylline may interact unfavorably with the negative carbonyl oxygen of the ester.

Structure 28-IV is one of several possible structures for the methyl <u>trans</u>-cinnamate-theophyllinate complex. Notice the roughly 180° reversal of the five- and six-membered rings relative to the first three structures. Our assumption here is that placement of the negative charge on the five-membered ring, which was the positive pole in the unionized molecule, has caused a reversal in the <u>sense</u> of the dipole, (though this is not assuredly so, because of the effects of counter-ions). Thus the nitrogen bearing the formal negative charge has been positioned over the relatively positive center of the aromatic ring. The two molecules are oriented in such a manner as to allow for favorable local interaction between the 2-oxygen of theophylline and the relatively positive carbonyl carbon of the ester.

Additional studies are certainly required to determine the validity of the general types of structures exemplified by Figure 28. The results presented in this work do not establish whether the ethylenic double bond actively participates in the binding interaction, or whether it merely serves to establish conjugation between the carboxyl group and the aromatic ring. The effect of complexation on the rate of addition across this double bond, as well as the effects of α- and β-substitution on K_{11}', might bear on this uncertainty. It would be interesting to observe changes in the nmr spectra of the ligand and substrate upon complexation; such a study may be experimentally unproductive because of the many non-equivalent protons present in both molecules. This experiment would have to be performed in D_2O for a valid comparison with aqueous solutions, and because of the low concentrations of substrate used in these studies (because of solubility limitations), a computer-average-time-scan would most certainly have to be employed. By using solvents other than water, it may be possible to find a solvent system in which the complex is relatively insoluble and separates as a crystalline solid. X-ray diffraction would then enable us to determine the structure of the solid complex. Although this structure may not be identical with that of the complex in solution, the general orientation of the two molecules would probably be similar. The continued use of the solubility, spectral, and kinetic

techniques for the determination of stability constants is recommended with inclusion of a fourth method, distribution between two immiscible phases, when one or two of the other techniques becomes experimentally unfeasible.

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APPENDIX

CONCURRENT CATALYTIC AND INHIBITORY EFFECTS BY IMIDAZOLE IN THE HYDROLYSIS OF ETHYL ACETATE

INTRODUCTION

Numerous reports have appeared in the literature concerning the catalytic effects of imidazole upon ester hydrolysis (1-8). Most of these catalytic effects have been observed near the neutral pH region in imidazole buffers, with the exception of a few esters such as phenyl acetate where catalysis was noted also in the alkaline region (7). Kirsch and Jencks have observed a very small catalytic effect for imidazole on the alkaline hydrolysis of ethyl acetate at neutral pH (8). In another publication these workers report that 1.0 M imidazole does not increase the alkaline hydrolysis rate of ethyl acetate in 0.024-0.050 M KOH (7). In this laboratory we have found a <u>decrease</u> in the observed rate of alkaline hydrolysis of ethyl acetate at high pH in the presence of 0.50 M imidazole. We attribute this to a non-specific solvent effect by imidazole on the activity of the ester and/or hydroxide ion. It is reasonable to assume that this solvent effect is present also at neutral pH, and yet a catalysis is observed at neutrality. Thus it is the purpose of this study to demonstrate how one must carefully investigate rate effects over a wide range of concentrations of the reactants involved. Failing to do so can lead to erroneous conclusions as to the magnitude and direction of certain effects on reaction rates.

RESULTS

Rate determinations were made by the pH-stat method, the details of which have been described elsewhere (9). From a series of six determinations, the second-order rate constant for the alkaline hydrolysis of ethyl acetate at 25.0° and ionic strength 1.0 was found to be $0.154 \pm 0.012\ M^{-1}sec^{-1}$.* This value is consistent with that found in the literature (10). A plot of k_{OH} *vs.* imidazole concentration is reasonably linear as shown in Figure A-1, and Table A-1 presents the data.

*Expressed as a mean ± 1 standard deviation.

TABLE A-I

Second-Order Rate Constants for Ethyl Acetate Alkaline Hydrolysis in the Presence of Imidazole at 25° and Ionic Strength 1.0

k_{OH} ($M^{-1}sec^{-1}$)[a]	Imidazole (M)
0.140, 0.145	0.10
0.142	0.20
0.138	0.25
0.138	0.30
0.132	0.40
0.123, 0.125	0.50

[a]All second-order rate constants are based on activity of OH^-.

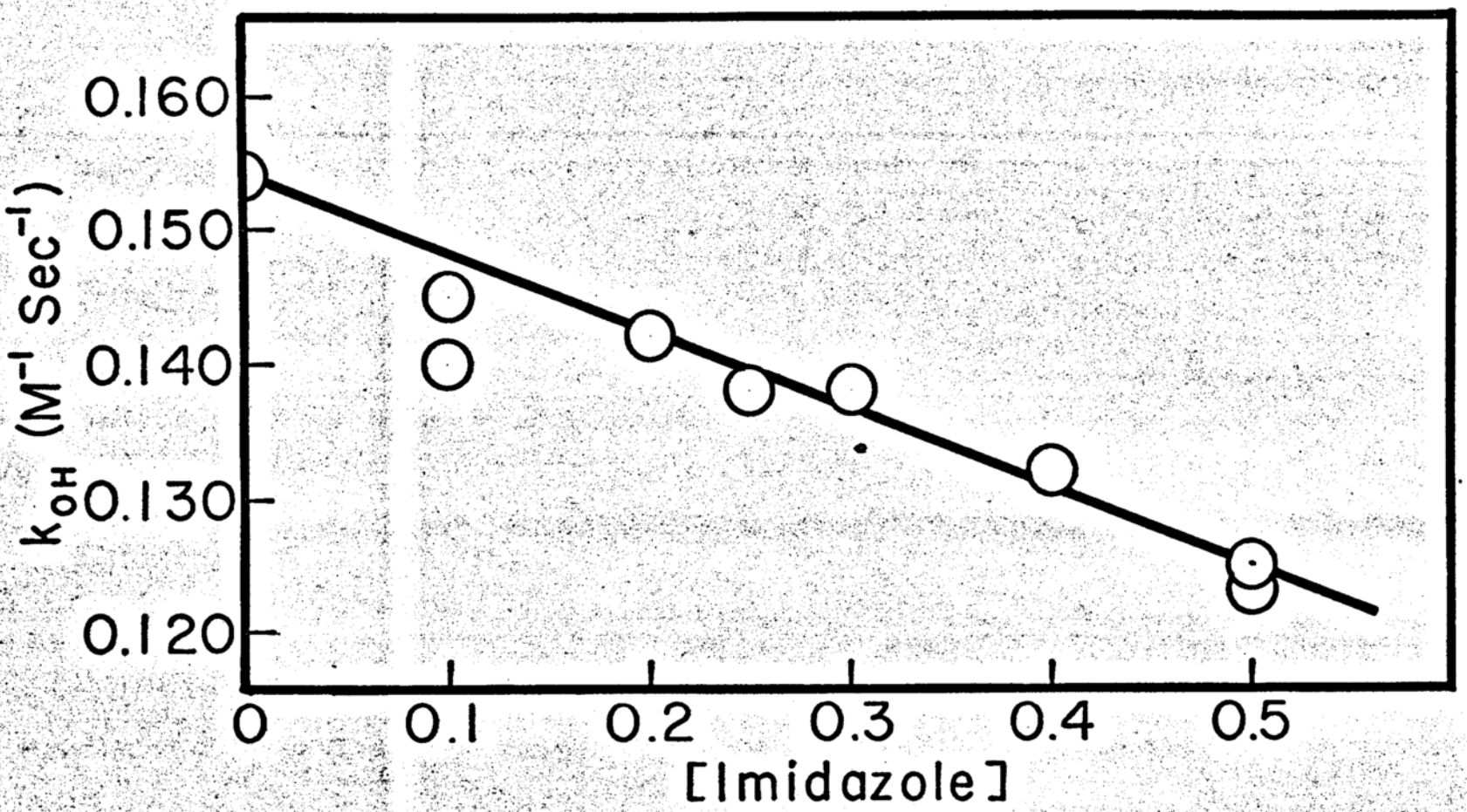

Figure A-1. Variation of observed second-order rate constant for the alkaline hydrolysis of ethyl acetate with molar concentration of imidazole at pH 12.30 and 25°.

DISCUSSION

Kirsch and Jencks have presented evidence that the imidazole catalyzed hydrolysis of ethyl acetate at neutral pH is a general base catalytic effect (8). Ethyl acetate also undergoes specific hydroxide-ion catalyzed hydrolysis. Thus the following reaction schemes should apply at pH 7 and above.

$$E + OH^- \xrightarrow{k_{OH}} P$$
$$E + I \xrightarrow{k_I} P \qquad \text{(A-1)}$$

The following empirical equation is formulated to define the line in Figure A-1.

$$k_{OH} = k^o_{OH} + m(I) \qquad \text{(A-2)}$$

where,

k_{OH} = observed second-order rate constant in presence of imidazole

k^o_{OH} = second-order rate constant in absence of imidazole

m = slope of straight line in Figure A-1

(I) = molar concentration of unionized imidazole.

Inserting the values determined for k^o_{OH} and m, equation (A-2) becomes,

$$k_{OH} = 0.154 + 0.058\,(I) \qquad \text{(A-3)}.$$

Assuming reaction scheme (A-1) applies, the following rate equation can be written.

$$v = k_{OH}(E)(OH) + k_I(E)(I) \qquad (A-4)$$

$$k_{obs} = \frac{V}{(E)} = k_{OH}(OH) + k_I(I) \qquad (A-5)$$

Substituting from (A-3) into (A-5):

$$k_{obs} = [0.154 - 0.058(I)](OH) + k_I(I) \qquad (A-6)$$

k_I has been determined to be 1.25×10^{-7} $M^{-1}sec^{-1}$ (8). Thus,

$$k_{obs} = [0.154 - 0.058(I)](OH) + 1.25 \times 10^{-7}(I) \qquad (A-7)$$

Using equation (A-7), we can calculate k_{obs} at various imidazole concentrations and pH's. For example, at pH 7 and with no imidazole present, k_{obs} is calculated to be 1.54×10^{-8} sec^{-1}. In the presence of 0.5 M imidazole at pH 7, $k_{obs} = 7.50 \times 10^{-8}$ sec^{-1}. Thus a catalytic effect of imidazole on ethyl acetate is observed. At pH 12, in the absence of imidazole, $k_{obs} = 1.54 \times 10^{-3}$ sec^{-1}. With 0.5 M imidazole, $k_{obs} = 1.25 \times 10^{-3}$ sec^{-1}. Thus at pH 12 a retardation in the hydrolytic rate is found. Thus either a net acceleration or a net retardation may be caused by imidazole depending on the relative magnitudes of k_I, m, and (OH).

This discussion leads to another case in point, that in which one would fortuitously choose reaction conditions at which no difference in the rates of hydrolysis would be observed in the presence or absence of imidazole. That such conditions are a function of pH reduces the possibility that one might randomly choose them. However, realizing that there is always experimental uncertainty associated with kinetic determinations, it is certainly possible that one might choose conditions at which the observed differences were so small that they would be dismissed as experimental errors.

The conclusion to be drawn from this study is that caution must be used in designing and interpreting catalytic studies. We have shown that varying degrees of catalysis or inhibition can be exhibited by imidazole merely by varying the pH (aside from the ionization equilibrium).

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