KINETIC INVESTIGATION OF THE STRUCTURES OF MOLECULAR COMPLEXES IN SOLUTION: BEHAVIOR OF SOME XANTHINE COMPLEXES

by

HONORE STELMACH

A thesis submitted in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

(Pharmacy)

at the

UNIVERSITY OF WISCONSIN

1969

"In the mountains of truth, you never climb in vain. Either you already reach a higher point today or you exercise your strength in order to be able to climb higher tomorrow!"

Friedrich Nietzsche

With this thought
I dedicate this thesis
to

LEONA and PETER

ACKNOWLEDGEMENTS

I wish to extend thanks to Dr. Kenneth A. Connors for directing me in this scientific investigation.

Additionally, I would like to express my gratitude to Dr. Stuart P. Eriksen, Dr. Joseph R. Robinson, and Dr. Charles A. Kelsey for their scientific stimulation which has enlarged the scope of my research interests. I would also like to thank Miss Michaelene Kedzierski and Mr. Stephen Hannon for technical assistance.

Partial support from the Public Health Service (Grant T1 CA 5104-04) and the National Institutes of Health (General Research Support Grant FR 05456) is acknowledged.

TABLE OF CONTENTS

TABLE OF CONTENTS - Cont.

I. INTRODUCTION

A. Molecular Complexes. Molecular complex formation is a reversible stoichiometric chemical reaction involving relatively weak forces of interaction in which the formation of the complex is very rapid. In fact, this process occurs so quickly that very few of these reaction rates can be measured by ordinary kinetic methods; therefore, these systems are considered to be at equilibrium. The general reaction for this equilibrium may be written

$$mS + nL \rightleftharpoons S_mL_n$$

where S represents the substrate (the substance whose apparent properties are measured) and L is the ligand or complexing agent. The stability of a complex is specified in terms of its stability (association, formation) constant and it may be written

$$K_{mn} = \frac{[S_mL_n]}{[S]^m\,[L]^n}$$

where K_{mn} is the constant applicable to the solvent system and temperature employed (1-4). These equations may also be utilized to describe metal coordination compounds where S is a metal ion or atom which can unite with one or more ligands; however, neither these compounds nor the inclusion compounds, in which one of

the constituents of the complex is trapped in the crystal lattice of the other, will be discussed in this paper. Complex formation will modify the possible energy states of the component molecules, and therefore can alter molecular properties. The presence of complexes sometimes can be inferred from the appearance of insoluble complexes; more frequently complexes are too unstable to be isolated in crystalline form, but exist only in solutions in equilibrium with their components, and then they can be detected from the nonadditive behavior in the chemical and physical properties of their solutions.

Molecular complexes can be utilized in pharmaceutical systems for solubilization of drugs or to suppress unwanted side reactions by decreasing the reactivity of a drug component (5,6). Caffeine and 1-ethyltheobromine have been shown to decrease the rate of hydrolysis of benzocaine and tetracaine in aqueous solutions (7-9). Caffeine and 8-chlorotheophylline demonstrate a marked stabilizing effect on the decomposition of riboflavin and isoalloxazines (10,11). These effects have been attributed to the steric and electronic influence of the component causing the complexing; such rate inhibitions are not as readily accounted for as have been the inhibitions and catalyzes observed for the alpha- and beta-cyclodextrins (12,13, and previous

papers). The cyclodextrins form solid inclusion compounds and complexes in solution, with the extent of complexing of the substrate molecule and the degree of ionization of the hydroxyl groups on the cyclodextrins determining the direction and magnitude of the rate effects. Complexes can influence not only stability and appearance of pharmaceuticals but also their pharmacological action. Levy and co-workers have demonstrated a decrease in the gastric absorption of salicylamide and salicylic acid upon complex formation with caffeine (14-18). In another study 8-nitrotheophylline was shown to bind to bovine serum albumin with considerable specificity. The pH binding profile indicated the presence of at least three classes of binding sites; two of the sites were nonspecific in binding ability and significantly bound all of the anions examined, while the third site had an affinity for the xanthines and the aromatic acids (19).

The preceding paragraph has pointed out some of the practical ways in which complexes might be utilized in physical-chemical systems. Undoubtedly the greatest current interest in complexation is the potential of complexes to serve as effective models for enzyme systems. The catalytic effects and specificity of enzymes are presumably dependent upon the formation of an enzyme-substrate complex. In this complexed state the proximity and geometry of the substrate and enzyme

are such that very efficient intramolecular reactions can occur. In addition, it is possible that electronic perturbations can be induced in the substrate in the complexed state so that it becomes more reactive to intermolecular reactions. With this in mind, studies of the complexation of relatively simple nonenzymatic materials in aqueous solution have been undertaken to determine the steric and electronic requirements of complex formation and reactivity, and some of these complexed systems now serve as interesting models for actual enzyme systems (13,20-22).

B. Forces of Interaction. It was proposed in 1929 that the formation of complexes was due to covalent bonding between the two components (23); however, abundant evidence exists to show that the intermolecular separation is usually greater than 3 A (24) and the forces of coordination are much weaker than those of covalent bonds (3). The exact nature and distribution of the intermolecular forces between the components in a molecular complex is still a matter of controversy, but it is generally accepted that the forces involved may include hydrogen bonding, van der Waals forces, and charge-transfer. If one of the components of a complex contains a hydrogen atom bonded to oxygen or nitrogen and the other a strongly electronegative atom such as

fluorine, oxygen, or nitrogen, then an electrostatic type of union known as a hydrogen bond may form and contribute to the stability of the complex. Van der Waals forces are due to interactions of three types: (1) simple electrostatic interactions or dipole-dipole forces, such as proposed by Higuchi and Lach for complexes of caffeine with sulfonamides or barbiturates (24-26); (2) forces due to electrostatic polarization of a second molecule, or dipole-induced dipole forces, as seen in the complex between nitrobenzene and naphthalene (2); and (3) forces due to instantaneous electrostatic attraction due to electron correlation, commonly called Heitler-London dispersion forces (27). The theory of charge-transfer forces is due mainly to Mulliken (4,28,29), who regards a loosely bound molecular complex as a resonance hybrid receiving a contribution from a no-bond structure and a contribution from a dative structure in which an electron has been transferred from the donor to the acceptor. Solvent orientation effects may also be important; dielectric effects of the solvent and recently the competing equilibria between the solutes and the solvent (30) have been shown to play a part.

C. Solvent and Structural Effects. Another factor of some importance in the formation of molecular complexes is the steric requirements. A charge-transfer complex

would exhibit maximum stability with maximum overlap of the π donor-π acceptor orbitals, such as is seen with chloranil-alkylbenzene complexes (31). The approach and close association of the donor and acceptor molecule is governed also by steric factors, i.e., if a substituent disrupts coplanarity and conjugation, then the ability to form complexes is more reduced than in the case where the steric effect just blocks off functional groups (24, 2). A good case in point is the work of Nakano and Higuchi (32). They found complexing between benzamide and theophylline, but remarkably less with N,N-dimethylbenzamide and theophylline. They postulate that the reduction in associative ability is due to the nonplanarity of the N,N-dimethylbenzamide (benzamide and theophylline are both planar). Connors and co-workers (33) have given a quantitative demonstration of the relationship between planar overlap and complex stability. Plotting the standard unitary free energy change for complex formation against the estimated planar overlap of the component with the smaller area, they obtain a linear relationship for various substrates with theophylline and with some other substances.

Addition of substituents, besides affecting coplanarity, can also affect the electron distribution within the molecule. Methyl trans-cinnamate, a polar molecule, has its negative pole directed towards the carboxylate portion of the molecule and its positive pole

in the vicinity of the aromatic ring, with a dipole moment of 1.95 D (34). If a nitro group is placed in the para position of the aromatic ring (methyl p-nitro-cinnamate), its group moment very nearly directly opposes that of the cinnamate and is approximately twice as large (35). Thus, upon addition of a p-nitro group "the sense of the moment is reversed in the p-nitro ester (relative to the unsubstituted ester)" (36). Donbrow and Jan (37) have studied the change in complex stability of caffeine-benzoic acid system upon changing substituents on the benzoic acid. The binding strength was found to decrease in the order p-OH > p-OMe > H > p-NO_2 and o-OH > o-OMe > H > o-Ac > o-NO_2. In general the introduction of a substituent that increases the electron density of an aromatic ring will enhance the stability of the complex provided that the substituent does not cause unfavorable steric effects (2, 38). A linear Hammett plot was obtained for the logarithms of the stability constant vs σ of the para compounds in the study quoted. They postulate the carbonyl group of the benzoic acid interacting with a part of the caffeine molecule by means of van der Waals forces or by a donor-acceptor mechanism and the aromatic ring involved in a donor-acceptor interaction (37). This treatment seems to favor caffeine as an electron acceptor.

The solvent can influence the stability of complexes. In the p-hydroxybenzoic acid-PVP system, increasing the phosphate buffer concentration from 0.1 - 0.3 molar resulted in an increase in complex stability at low PVP concentration. An increase in the ionic strength from 0.2 - 0.5 with sodium chloride caused an increase in the complexing tendency for all concentrations of PVP (39). In the N'-methylnicotinamide (cation)-8-chloro-theophylline (anion) system the degree of complexing varied with the salt used, being much greater when sodium acetate was used than when sodium perchlorate was used (40). Two main mechanisms have been proposed to account for these observations: (1) a "salting out" or de-solvation of organic groups and molecules (39, 41); (2) a more or less direct interaction between the ions of the salt and the compounds of the complex (40, 41). Another solvent property to be taken into consideration is the dielectric constant of the medium. Ross and Labes (42) studied the effect of increased ethanol concentration on the complexing of naphthalene with 1,3,5-trinitrobenzene in ethanol-chloroform and found that as the dielectric constant increased, the stability constant decreased. They attribute this to preferential stabilization by solvation of either naphthalene or 1,3,5-trinitrobenzene rather than the complex. Similar solvent effects were investigated using proton magnetic resonance (30).

D. Complex Structure. It has not yet been possible to determine the structure of nonisolable complexes such as the xanthine-cinnamate complexes (43, 44, 33). From crystalline complexes such as s-trinitrobenzene with p-iodoaniline (45, 46) it has been observed that the mode of association often is that of vertical stacking of the donor-acceptor rings in a partial overlapping fashion favorable to π electron interactions (31). Generally the interplanar distances are about 3.4 A (3) and thus the interactions must be relatively weak, with little or no disruption of the covalent bonds of the components. It is unlikely that the crystalline complex differs greatly from the complex in solution with respect to molecular dimensions (47); however, one cannot assume that the structure of the complex is the same. For example, benzene-halogen complexes are known to interact in a 1:1 stoichiometry in solutions, but a coordination number of greater than one is implied by the X-ray data of these same crystalline benzene-halogen complexes (3).

In spite of these difficulties several attempts have been made to determine the structure of xanthine complexes and it is these to which we shall confine our attention. Pullman and Pullman used quantum mechanical calculations and found that caffeine and other xanthines should act as good electron donors (48). Schnarre and Martin used a similar mathematical approach to calculate

the formal π charge for the atoms in caffeine and benzocaine (49). They felt that the specific orientation of the two molecules is controlled by several electrostatic interactions and possible localized charge-transfer interactions. On this basis they proposed the following structure, Figure 1, in which the distances between the various reactive centers of the two molecules are consistent with the Stuart-Briegleb models of benzocaine and caffeine. The structure is quite different from the one proposed by Eckert (50); however, Shefter points out that "there has been no experimental evidence reported in the literature to substantiate such a mechanism" (54).

Besides the mathematical approach, a few structural studies have been carried out on crystalline xanthine complexes. The crystalline structure of tetramethyl uric acid-pyrene has been published (51) and shows only a small degree of π molecular orbital overlap. Hence the authors attribute the stability of the complex to van der Waals forces. The 8-ethylhypoxanthine-5-fluorouracil complex studied by Kim and Rich is held together by hydrogen bonds in the molecular plane, while van der Waals forces hold the molecules of the lattice together (52). The caffeine-5-chlorosalicylic acid complex was studied by Shefter, who found that the caffeine molecule showed significant deviations from planarity. Intermolecular hydrogen bonds were found to

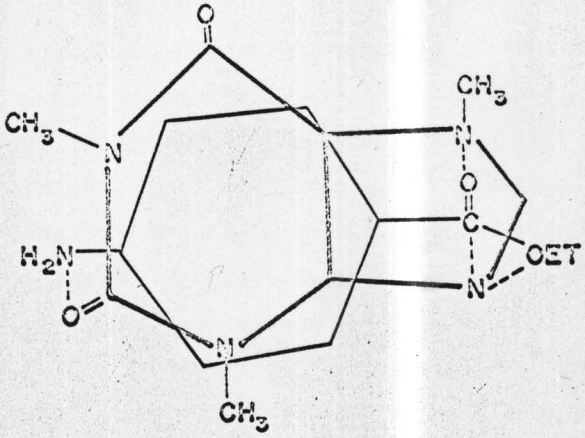


Figure 1. Orientation of the caffeine-benzocaine complex proposed by Schnaare and Martin (49).

hold the molecules of the lattice together with polarization bonding also playing a dominant role in the a stacking of the molecules (53). The structure is given in Figure 2 (54). Arnone and Marchessault have investigated the crystal structure of the caffeine-pyrogallol complex, Figure 3 (55). The major forces of interaction in this crystal are van der Waals forces; no significant hydrogen bonding was found between the molecules and, as with the caffeine-5-chlorosalicylic acid complex (53), charge-transfer forces are very weak if present at all (55). It is necessary to emphasize once again that the complex in solution does not necessarily possess the same stoichiometry or structure.

Solution studies of xanthines with cinnamates and related compounds have provided us with several observations and inferences bearing on the structures of these complexes:

(1) The area correlation described earlier suggests that the complex forms with the two molecular planes parallel, or very nearly so (33).

(2) The relative insensitivity of complex stability to changes in X in the series $C_6H_5CH=CH-COX$ implies that there is little or no direct interaction with the X group in the complex (36, 33).

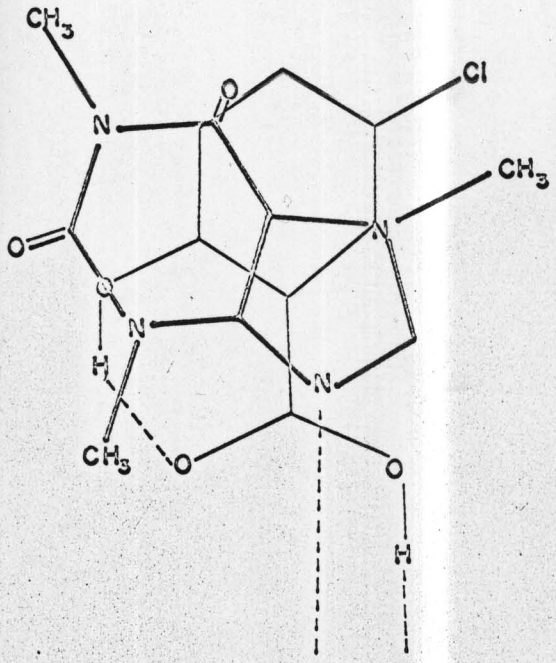


Figure 2. Structure of caffeine-5-chlorosalicylic acid complex as determined by X-ray crystallographic analysis: Shefter (53, 54).

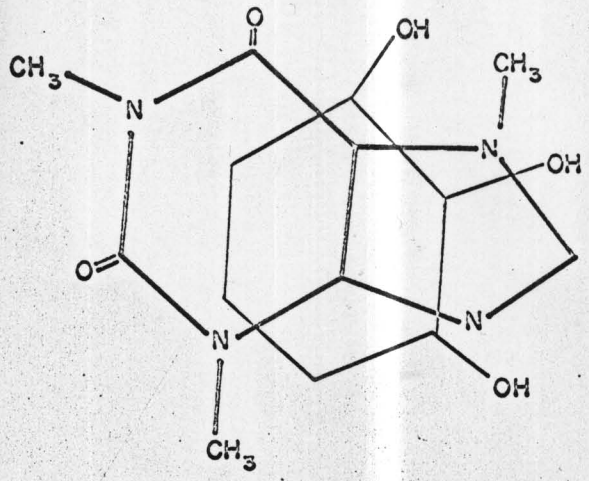


Figure 3. Structure of caffeine-pyrogallol complex as determined by X-ray crystallographic analysis: Arnone and Marchessault (55).

(3) $C_6H_5CH=CH-COX$ in the complexed form displays essentially no reactivity to hydroxide attack. This observation suggests that the xanthine must be very close to the carbonyl group (44, 56b).

(4) In a compound $C_6H_5CH=CH-COOR$, sulfite addition to the double bond was essentially completely inhibited by complex formation, implying that the xanthine is very close to the double bond (44).

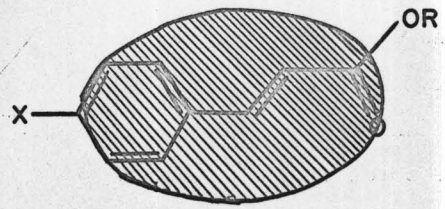
(5) Alteration of the phenyl group in $C_6H_5CH=CH-COOMe$ to methyl causes a marked decrease in complex stability, suggesting that the ring is directly involved in complexation (33, 36).

E. Research Plan. It is possible to utilize quantitative measures of solution properties to demonstrate complex formation and to study the stability and properties of complexes. These physical methods have already been described (38, 2-4), along with the equations for utilization of the data (57, 1, 44, 58). Besides the changes in physical properties, the components of a complex may also show a change in chemical reactivity due to the close association of a second component.

From the experimental data on the effects of complexation on the alkaline hydrolysis of carboxylic acid derivatives (see above), it was felt that judicious

choice of substrate and ligand should allow us to deduce the general regions of molecular overlap between the substrate and ligand and, perhaps, to elucidate the solution structure of a molecular complex. Previous studies (56a, 44, 1) suggested that the kinetic method of analysis would be the most powerful tool as it would provide non-equilibrium information about a system that is at equilibrium with respect to complex formation. Where desirable the spectral and solubility methods were also utilized to obtain information on complex stability.

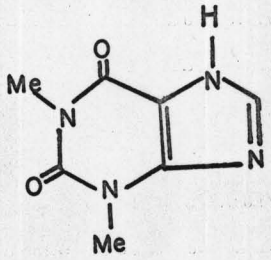
It was desirable to choose substrate and ligand molecules that are essentially planar to facilitate maximum overlap, and to select molecules that undergo 1:1 complexation. The initial substrates chosen were the acetoxycinnamic acids and their methyl esters. Crystallographic studies have shown that p-hydroxycinnamic acid is "almost planar" (59) and m-bromocinnamic acid is planar (60). Structure 1 shows a cinnamic acid derivative; the shaded area has been postulated to be involved in the xanthine-cinnamate complex (see solution



1

inferences (2) - (5) above). The acetoxycinnamic acids are substituted phenyl acetates and the hydrolysis reaction will proceed at a rate convenient to utilize the kinetic method of analysis. The labile ester group is at the opposite end of the molecule from all cinnamates previously used. The variation in complexing tendency and in the hydrolytic activity of the complexed ester should show the extent to which the ligand overlaps the phenyl ring in the complex, and perhaps the alteration in the reactivity of the system could be interpreted in terms of a probable orientation of the substrate and the ligand.

Theophylline (2) and related xanthines were chosen as ligands. Theophylline is planar (53, 61) and does not



2

dimerize in solution (61); however, it has been shown that caffeine (62, 63) and 7-propyltheophylline (61) are capable of self-aggregation. No partition studies of the other xanthines utilized here have been carried out;

however, due to their large solubility in aqueous solvents, self-aggregation is considered to be a possibility. These xanthines were felt to be useful in observing overall effects, even though it may not be possible to interpret the results in terms of individual reactivities for each of the complexes present.

The effect of theophylline on m- and p-substituted methyl cinnamates was studied. As the substituent is changed from the electron-donating, $-OCH_3$, to the very electron-withdrawing, $-NO_2$, the ability of the methyl cinnamate to participate in electronic interaction with theophylline should be markedly altered. A correlation of stability constant with the Hammett substituent constant should indicate a well-behaved polar effect. Marked deviations will suggest a disturbing influence, either direct interaction between the substituent and theophylline or a change in the structure of the complex. Either case will provide information about the structure of the complex.

In addition to the structure-reactivity studies, some medium effects were investigated, the effects of ionic strength, cations and anions, and mixed solvents being studied with neutral complexes in a preliminary study.

EXPERIMENTAL

A. Chemicals. o-Acetoxycinnamic acid was prepared by heating o-hydroxycinnamic acid (K & K Laboratories) with sodium acetate and acetic anhydride for two hours on a steam bath. The ester was purified by recrystallization from boiling water and then from ethanol; mp 145° (lit. 146° (64)). m-Acetoxycinnamic acid was prepared by treating m-hydroxycinnamic acid (Aldrich Chemical Co.) in 15 molar sodium hydroxide with acetic anhydride. The ester was purified by recrystallization from aqueous ethanol and then from boiling water; mp 151-151.5° (lit. 151° (65)). p-Acetoxycinnamic acid was prepared by treating p-hydroxycinnamic acid (K & K Laboratories) in 15 molar sodium hydroxide with acetic anhydride. The ester was recrystallized from aqueous ethanol; sublimes 195° (lit. sublimes 196° (66)).

Methyl trans-cinnamate (Matheson Coleman and Bell) was distilled under reduced pressure; bp 91-94°/2-3 mm, mp 33-34° (lit. mp 33.4-34.5° (67)). Methyl p-acetoxycinnamate was prepared by a two-step process: First methyl p-hydroxycinnamate was prepared by refluxing p-hydroxycinnamic acid with methanol for about ten hours in the presence of a small amount of sulfuric acid. The ester was purified by recrystallization from boiling water; mp 136.5-137° (lit. mp 137° (43)). This monoester,

in 15 molar sodium hydroxide, was then treated with acetic anhydride. The product, methyl p-acetoxycinnamate, was recrystallized from boiling water; mp 82-83°. Analysis for $C_{12}H_{12}O_4$: Calcd., C = 65.45%; H = 5.49%. Observed, C = 65.51%; H = 5.48%. Methyl m-acetoxycinnamate and methyl o-acetoxycinnamate were prepared by the same procedure as the corresponding para derivative. Methyl m-acetoxycinnamate was recrystallized from aqueous ethanol; mp 60-62°. Analysis for $C_{12}H_{12}O_4$: Calcd., C = 65.45%; H = 5.49%. Observed, C = 65.44%; H = 5.49%. Methyl o-acetoxycinnamate was recrystallized from aqueous ethanol; mp 42.0-43.5°. Analysis for $C_{12}H_{12}O_4$: Calcd., C = 65.45%; H = 5.49%. Observed, C = 65.50%; H = 5.50%.

p-Hydroxybenzalacetone was synthesized according to the procedure of Buck and Heilbron (68) and was recrystallized from boiling water; mp 102-103° (lit., mp 102-103° (69)). p-Acetoxybenzalacetone was obtained by treating p-hydroxybenzalacetone in 15 molar sodium hydroxide with acetic anhydride. The ester was recrystallized from aqueous methanol; mp 67.5-68.5°. Analysis for $C_{12}H_{12}O_3$: Calcd., C = 70.57%; H = 5.92%. Observed, C = 70.73%; H = 5.91%.

Phenyl acetate (Aldrich Chemical Co.) was purified by distillation; the fraction with boiling point 194-197° was utilized (lit., 194° (70)). p-Acetylphenyl acetate was prepared according to the procedure of Chattaway (71) (as were the following four substituted

phenyl acetates) and purified by recrystallization from aqueous ethanol; mp 45-46.5° (lit., mp 54° (72)). p-Chlorophenyl acetate was purified by distillation; bp 122°/3 mm (lit., bp 226-228° (73)). p-Methylphenyl acetate was purified by distillation; bp 60°/1 mm (lit., bp 212-213° (73)). o-Nitrophenyl acetate was recrystallized from ethanol; mp 38-38.5° (lit., mp 36-38° (74)). p-Nitrophenyl acetate was recrystallized from Skellysolve B; mp 78° (lit., mp 82° (74)). Methyl p-nitrocinnamate was prepared by Dr. B. J. Kline of this laboratory, mp 163°. Methyl p-methoxycinnamate was prepared according to the procedure of Vogel for aromatic esters (75) and purified by recrystallization from aqueous ethanol (as were the following four substituted methyl cinnamates); mp 86.5-87.5° (lit., mp 88-89° (76)). Methyl p-chlorocinnamate; mp 74°. Methyl p-methyl-cinnamate; mp 56.4° (lit., mp 57-58° (77)). Methyl m-hydroxycinnamate; mp 85.0-86.5° (lit., mp 85° (65)). Methyl m-bromocinnamate; mp 52° (lit., mp 55-56° (59)). Methyl m-methoxycinnamate was purified by distillation under vacuum; bp 114°/1 mm (lit., bp 305-307°/748 mm (79)).

7-(2,3-Dihydroxypropyl)-theophylline (Aldrich Chemical Co.) was recrystallized from ethanol; mp 164-165° (lit., mp 155° (73)). This compound was found to be stable for 16 hours in aqueous solution at a pH of 10.85. Theophylline (Merck & Co., U.S.P.) was

recrystallized from water. It was then dried at 150° for twelve hours; mp 271-272°. 8-Bromotheophylline, 8-chlorotheophylline, and 8-iodotheophylline were purified as described elsewhere (44). Caffeine (U.S.P.) was recrystallized from water; mp 235-236°. Theophylline 7-acetic acid, acetonitrile, and isooctane were purified as described by Mollica and Connors (56). Naphthalene, resublimed (J. T. Baker Chemical Co.) was used as received.

7-(n-Propyl)-theophylline was prepared according to a modified Schwabe (80) process; 0.277 moles of theophylline were added to a flask containing 0.277 moles of potassium hydroxide dissolved in 175 ml of ethanol. The flask was fitted with a condenser and heated with the addition of a minimal amount of water required to effect solution. 0.30 Moles of n-propyl iodide, which had been decolorized by extraction with an aqueous sodium thiosulfate solution, was added to the theophyllinate solution and the solution was refluxed for six hours. The solution was evaporated to dryness on a rotary evaporator and the residue was extracted with hot chloroform. After evaporating the chloroform, the compound was recrystallized from boiling water; mp 99-102° (lit., mp 99-100° (80)).

To prepare 7-(3-hydroxypropyl)-theophylline a mixture of 0.75 mole of theophylline, 375 ml of water, and 1.125 moles of 3-chloro-1-propanol (Aldrich Chemical Co.) was placed in a round bottom flask fitted with a

reflux condenser and heated to boiling. The boiling solution was treated with a solution of 1.125 moles of sodium hydroxide in 90 ml of water over a period of three hours. After the addition of the sodium hydroxide was completed, the refluxing was continued for one hour. The mixture was then evaporated to dryness on a rotary evaporator. The residue was extracted with 750 ml of boiling anhydrous ethanol and filtered. The solid that crystallized out was filtered and recrystallized twice more from anhydrous ethanol; mp 150-151.5° (lit., mp 149-150° (78)).

7-(2-Hydroxypropyl)-theophylline was synthesized according to the procedure of Zelnick, et al. (81); 0.3 mole of theophylline was dissolved in 175 ml of water containing 0.3 mole of sodium hydroxide. 23 ml of 1-chloro-2-propanol* were added to the reaction vessel and it was refluxed for seven hours. The mixture was evaporated to dryness on a rotary evaporator and the residue was extracted with hot absolute alcohol. The compound crystallized out upon standing and required a

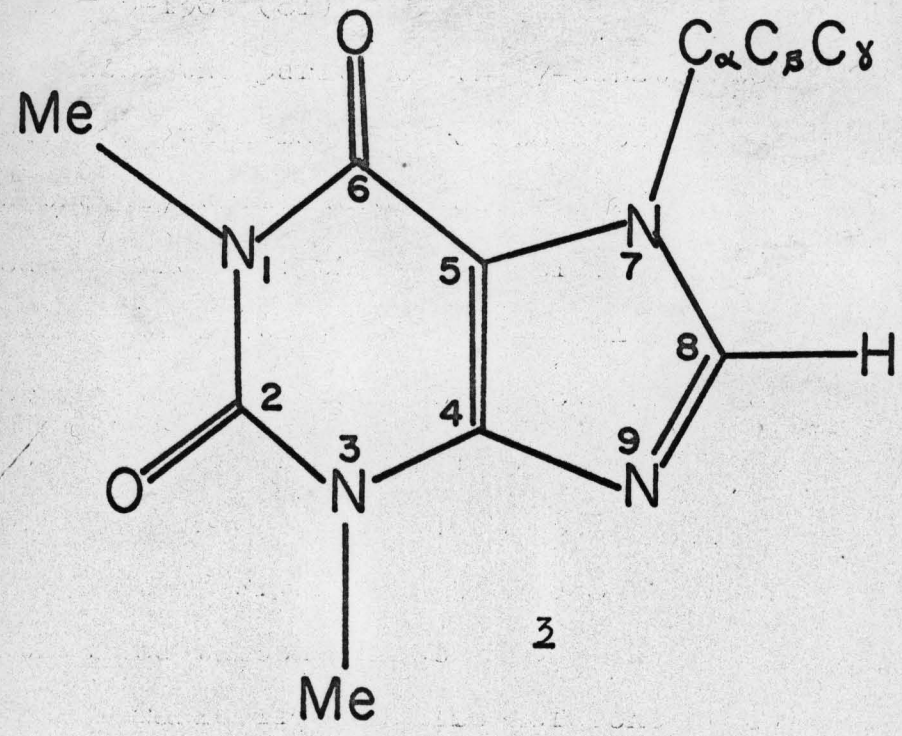
---

*The only 1-chloro-2-propanol (Eastman Organic Chemicals) available is contaminated with 25% 2-chloro-1-propanol. Purification requires two distillations through a 110 cm column filled with glass helices and then distillation through a Vigreux column (82, 83). Due to a limited supply of the alcohol, it was decided to purify the solid product rather than the volatile starting material.

minimum of twelve recrystallizations from hot absolute ethanol to obtain a satisfactory product; mp 132.5-133.5° (lit., mp 135-136° (81)).

To assess the purity of the 7-substituted theophyllines, thin-layer chromatography was carried out between the crystallizations until only one spot was obtained. Commercial thin-layer silica gel plates (Brinkman Instruments, Inc.) were utilized. The solvent was approximately 10% methanol - 90% chloroform. The R_f values obtained are given in Table I and compare favorably with literature values (84, 85).

NMR spectra (Figures 4, 5, and 6) were obtained for the synthesized theophyllines in D_2O and assignments were made in Table II; see also reference (86). (See structure 3.)



3

TABLE I

Thin-Layer Chromatography of Some Xanthines

Compound[a]	R_f[b]
7-(2,3-Dihydroxypropyl)-theophylline	0.15
7-(3-Hydroxypropyl)-theophylline	0.27
7-(2-Hydroxypropyl)-theophylline	0.35
Theophylline	0.36
Caffeine	0.48
7-(n-Propyl)-theophylline	0.53

[a] 7-Substituent numbering (see 3) is $1 \equiv \alpha$; $2 \equiv \beta$; $3 \equiv \gamma$.

[b] Silica Gel F-254 plates; precoated, abrasion resistant; 0.25 mm layer thickness distributed by Brinkman Instruments, Inc. The solvent was approximately 10:90::MeOH:$CHCl_3$.

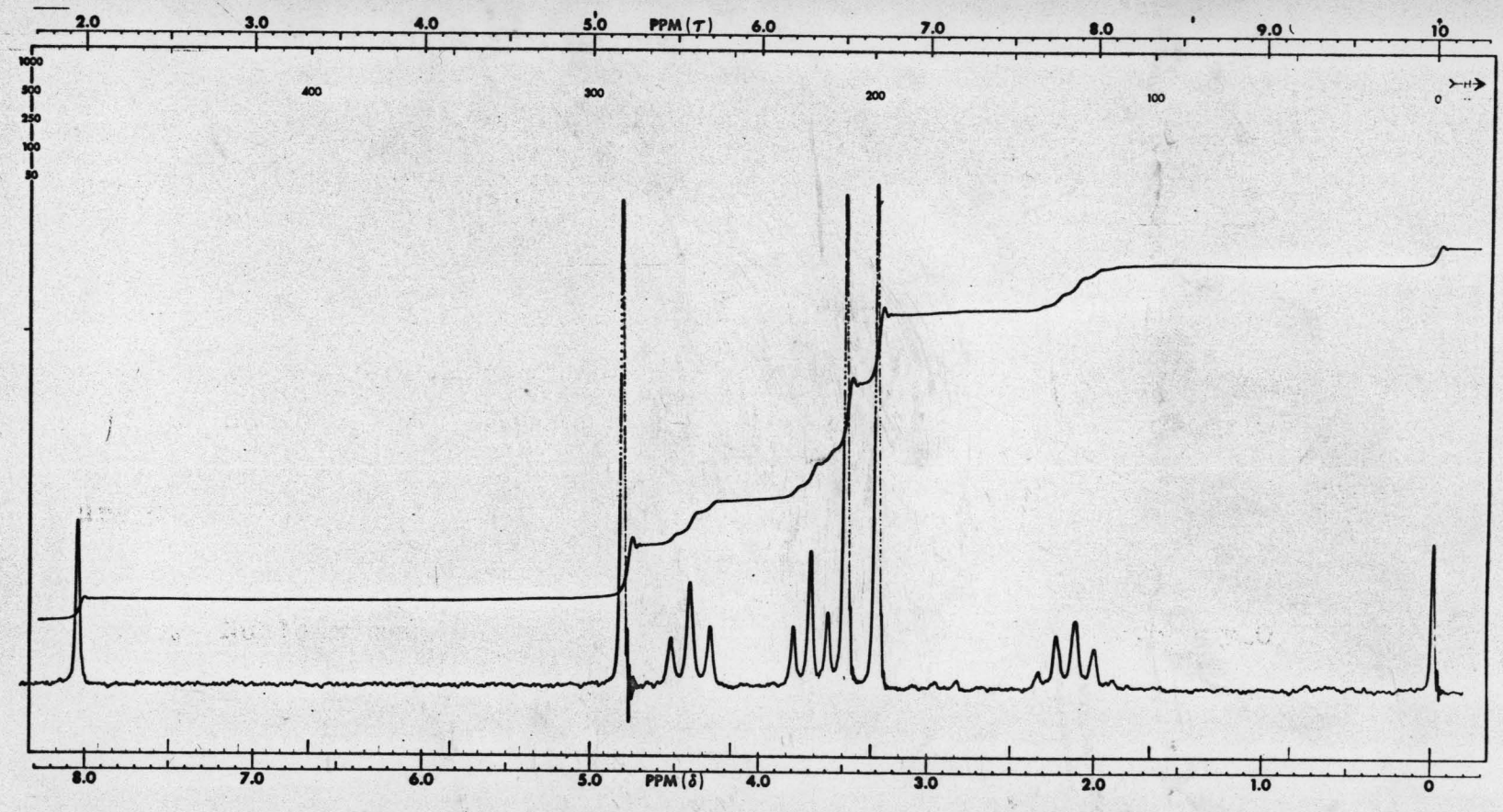


Figure 4. Nuclear magnetic resonance spectrum of 7-(3-hydroxypropyl)-theophylline in deuterium oxide: room temperature; sweep width 500 Hz.

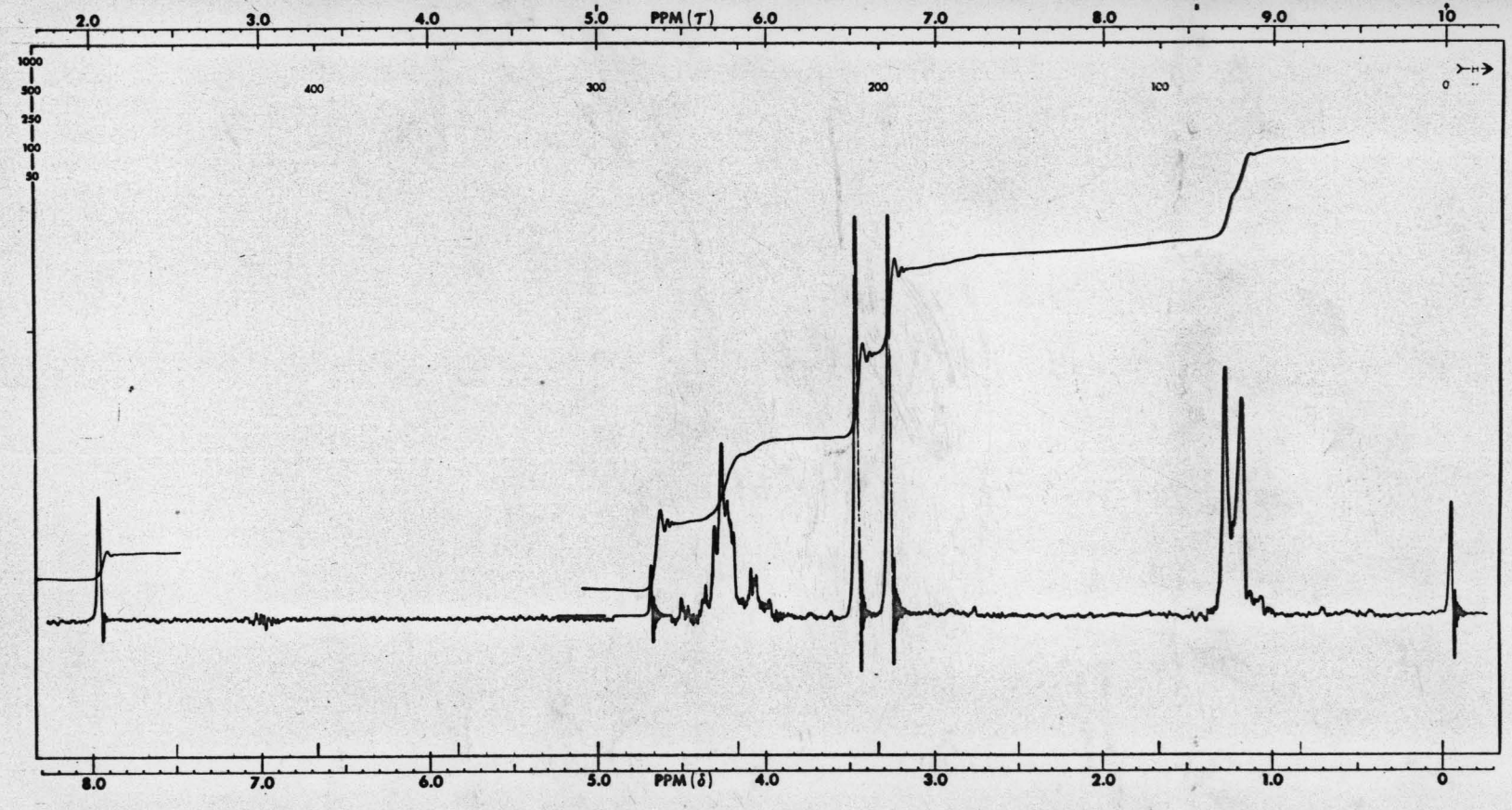


Figure 5. Nuclear magnetic resonance spectrum of 7-(2-hydroxypropyl)-theophylline in deuterium oxide: room temperature; sweep width 500 Hz.

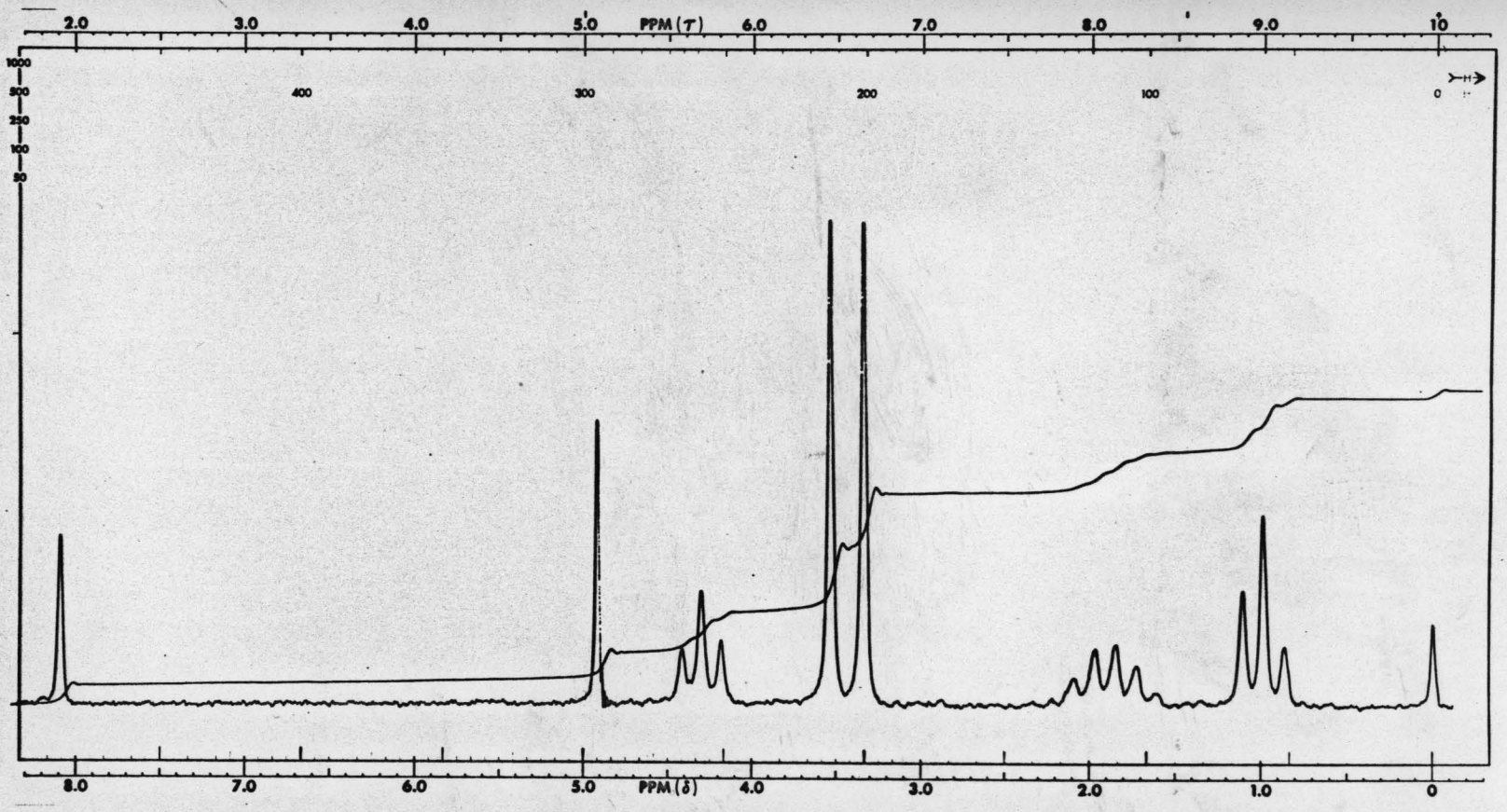


Figure 6. Nuclear magnetic resonance spectrum of 7-(n-propyl)-theophylline in deuterium oxide: room temperature; sweep width 500 Hz.

TABLE II

Assigned Chemical Shifts in Tau Units[a,b]

Protons[c]	7-(3-Hydroxypropyl)-theophylline	7-(2-Hydroxypropyl)-theophylline	7-(n-Propyl)-theophylline
1-Methyl	6.69	6.66	6.64
3-Methyl	6.48	6.47	6.46
8-H	1.94	1.99	1.90
7-Substituents			
Methylene doublet (α-H)		8.71	
Methylene triplet (α-H)	5.57		5.69
(γ-H)	6.29		
Methylene pentet (β-H)	8.87		
Methylene sextet (β-H)		5.57	8.08
Methyl doublet (γ-H)		5.70	
Methyl triplet (γ-H)			9.00

[a]Relative to tetramethyl silane.

[b]In D_2O.

[c]See structure 3 for numbering.

Reagent-grade inorganic chemicals were used without further purification. Standard buffers were prepared according to Bates (87); other buffers were prepared according to Bates and Bower (88) or Boyd (89). All solutions were prepared using freshly boiled redistilled (from alkaline permanganate in an all glass system) water.

The spectral data for the compounds described in this section are given in Tables III and IV with the structures given in Table V.

B. Apparatus. A constant temperature of 25.0°C for spectral and kinetic studies was maintained with water baths controlled to a precision of ±0.01°C by Sargent Thermonitor Relays. Solubility studies were made in a bath controlled to ±0.05°C by a mercury column thermoregulator (Brownwill Scientific Co.).

pH measurements and adjustments were at 25.0°C on a Sargent model DR pH meter equipped with a Sargent universal range glass-calomel combination electrode, S-30072-15, or on a Corning model 12 pH meter with an expanded scale using a Beckman Type E-3 wide range glass electrode. For measurements below pH 7 the meters were standardized between pH 4.008 and 7.413; for the alkaline region the meters were standardized between pH 7.413 and 9.180 using the standard buffers previously described.

TABLE III

Spectral Data of Substrates and Hydrolysis Products

Compound	λ_{max} (nm)	$\log \epsilon_{max}$	Solvent[a]
o-Acetoxycinnamic acid	267	4.35	pH 1.0 HCl
o-Hydroxycinnamic acid	233	3.36	pH 13.0 NaOH
m-Acetoxycinnamic acid	274	4.27	pH 1.0 HCl
m-Hydroxycinnamic acid	340	3.52	pH 13.0 NaOH
p-Acetoxycinnamic acid	279	4.41	pH 1.0 HCl
p-Hydroxycinnamic acid	333	4.36	pH 13.0 NaOH
Phenol	262	2.42	isooctane
p-Acetylphenol	324	4.30	pH 13.0 NaOH
p-Chlorophenol	316.5	2.80	pH 13.0 NaOH
p-Methylphenol	295	3.50	pH 13.0 NaOH
o-Nitrophenol	416	3.65	pH 13.0 NaOH
p-Nitrophenol	400	4.26	pH 13.0 NaOH
p-Acetoxybenzalacetone	292	4.36	pH 6.0 phosphate
p-Hydroxybenzalacetone	380	4.57	pH 13.0 NaOH
Methyl p-acetoxycinnamate	281	4.37	pH 6.0 phosphate
Methyl p-hydroxycinnamate	308	4.34	pH 6.0 phosphate
Methyl p-chlorocinnamate	285	4.39	pH 6.0 phosphate
Methyl p-methylcinnamate	288	4.36	pH 6.0 phosphate
Methyl p-methoxycinnamate	308	4.36	pH 6.0 phosphate
Methyl m-bromocinnamate	275	4.32	pH 6.0 phosphate
Methyl m-hydroxycinnamate	278	4.28	pH 6.0 phosphate
Methyl m-methoxycinnamate	278	4.32	pH 6.0 phosphate
Naphthalene	275	3.74	isooctane

[a]Aqueous solvents: ionic strength 0.3; 25.0°. Organic solvent: 25.0°.

TABLE IV

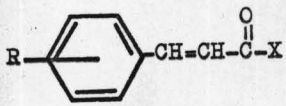
Spectral Data of Ligands

Compound	λ_{max} (nm)	$\log \epsilon_{max}$	Solvent
Theophylline	272	4.01	pH 5.6 acetate
Caffeine	273	4.00	water
7-(2,3-Dihydroxypropyl)-theophylline	273	3.96	water
7-(2-Hydroxypropyl)-theophylline	273	3.97	water
7-(3-Hydroxypropyl)-theophylline	273	3.97	water
7-(n-Propyl)-theophylline	273	3.98	pH 6.0 phosphate

TABLE V

Structures of Substrates and Ligands

Substrates[a]

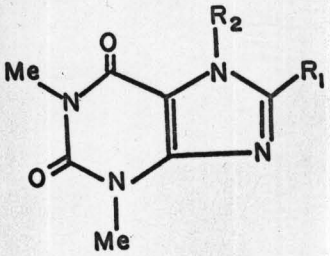


R	X	
CH_3COO-	HO-	Acetoxycinnamic acid
CH_3COO-	CH_3O-	Methyl acetoxycinnamate
CH_3COO-	CH_3-	Acetoxybenzalacetone
HO-	CH_3-	Hydroxybenzalacetone
O_2N-	CH_3O-	Methyl nitrocinnamate
CH_3-	CH_3O-	Methyl methylcinnamate
CH_3O-	CH_3O-	Methyl methoxycinnamate
HO-	CH_3O-	Methyl hydroxycinnamate
Br-	CH_3O-	Methyl bromocinnamate
Cl-	CH_3O-	Methyl chlorocinnamate

[a]All have the trans geometry about the olefinic double bond.

TABLE V - Cont.

Ligands



R_1	R_2	
H-	H-	Theophylline
H-	CH_3-	Caffeine
H-	$CH_3CH_2CH_2$-	7-(n-Propyl)-theophylline
H-	$CH_3CHOHCH_2$-	7-(2-Hydroxypropyl)-theophylline
H-	$CH_2OHCH_2CH_2$-	7-(3-Hydroxypropyl)-theophylline
H-	$CH_2OHCHCHCH_2$-	7-(2,3-Dihydroxypropyl)-theophylline
Cl-	H-	8-Chlorotheophylline
Br-	H-	8-Bromotheophylline
I-	H-	8-Iodotheophylline
CH_3O-	CH_3-	8-Methoxycaffeine
H-	CH_2ClCH_2-	7-(2-Chloroethyl)-theophylline
H-	$HOOCCH_2$-	Theophylline 7-acetic acid

Spectrophotometric measurements were made on a Cary Spectrophotometer, model 14, 15, or 16; models 14 and 15 are recording spectrophotometers. Each instrument was equipped with a thermostated cell compartment and a circulating water bath which was maintained at 25.0°±0.1°C. Nuclear magnetic resonance spectra were obtained with a Varian Model A60-A High Resolution Analytical Spectrometer.

Melting points were determined on a Thomas-Hoover Capillary Melting Point apparatus. A 2.00 ml microburet (Roger Gilmont Instruments) was used for accurate delivery of small volumes.

C. Procedures.

1. Solubility Measurements. Specific details for carrying out solubility determinations were reported by earlier workers and can be found elsewhere (56b); however, the general method is outlined here. Substrate in excess of its solubility was added to 10.0 ml of buffer solution containing varying concentrations of ligand, which were obtained by serial dilution of a stock solution of the ligand. The vials were sealed with Parafilm® in addition to the screw-cap, and were then tumbled in a constant temperature water bath until equilibrium was reached; usually 24 hours was sufficient.

Aliquots of the supernatant were withdrawn by a pipette, the tip of which had been wrapped in glass wool. Analysis of the sample was done by one of two methods, depending upon the spectral absorption of the substrate and the ligand. When the substrate absorbed light at a wavelength different from the ligand, the aliquot of the supernatant was diluted with an appropriate amount of solvent or buffer and the absorbance read directly. When the substrate and ligand both absorbed at the analytical wavelength, the substrate was extracted with isooctane and the isooctane phase was then measured spectrophotometrically, or, when necessary, diluted and then measured.

2. Spectral Measurements. The use of spectroscopic measurements to evaluate complexation equilibrium constants for systems similar to those encountered here has been described by Mollica and Connors (56b). Their methods were utilized here, with the addition of the substrate being made either with the microburet previously described or with self-leveling lambda pipettes.

In selecting the wavelength for this determination, it was preferable to have substrate of the same concentration in the reference cell and to read the change in absorbance directly (this condition also simplifies the subsequent spectral measurements). By

scanning the solution of the highest ligand-substrate concentration *vs* ligand, one can determine the wavelength of maximum change in absorbance. This optimal wavelength is often lower than would be chosen if the highest ligand-substrate were scanned against the buffer without an equivalent amount of substrate in the reference cell. The technique is shown by the example in Figure 7, with substrate data obtained given in Table VI.

3. *Kinetic Measurements*. The details of the spectrophotometric procedures used for kinetic measurements of reactions with various half-lives have been described (56b) and were followed in this study. Reactions were followed at 25.0° for about three half-lives unless the method of Guggenheim (90) was employed, in which case data were obtained over at least four half-lives. "Infinity" values were taken after ten half-lives. The second-order rate constants for alkaline hydrolysis of these esters are given in Table VII.

D. *Treatment of Data*. A theoretical comparison of the mathematical methods, assumptions, and approximations for the evaluation of stability constants by the three experimental methods has been made by Connors and Mollica (1). In addition, a new kinetic approach was tried (*vide infra*). The equations and plotting procedures are presented here. All calculations assumed

Figure 7. Ultraviolet preliminary scans for a spectral study: (A) Theophylline *vs.* buffer; (B) Methyl *p*-hydroxycinnamate *vs.* buffer; (C) Theophylline plus methyl *p*-hydroxycinnamate *vs.* buffer; (D) Theophylline plus methyl *p*-hydroxycinnamate *vs.* methyl *p*-hydroxycinnamate. T = 25.0°; phosphate buffer; ionic strength 0.3; 1.6% CH_3CN.

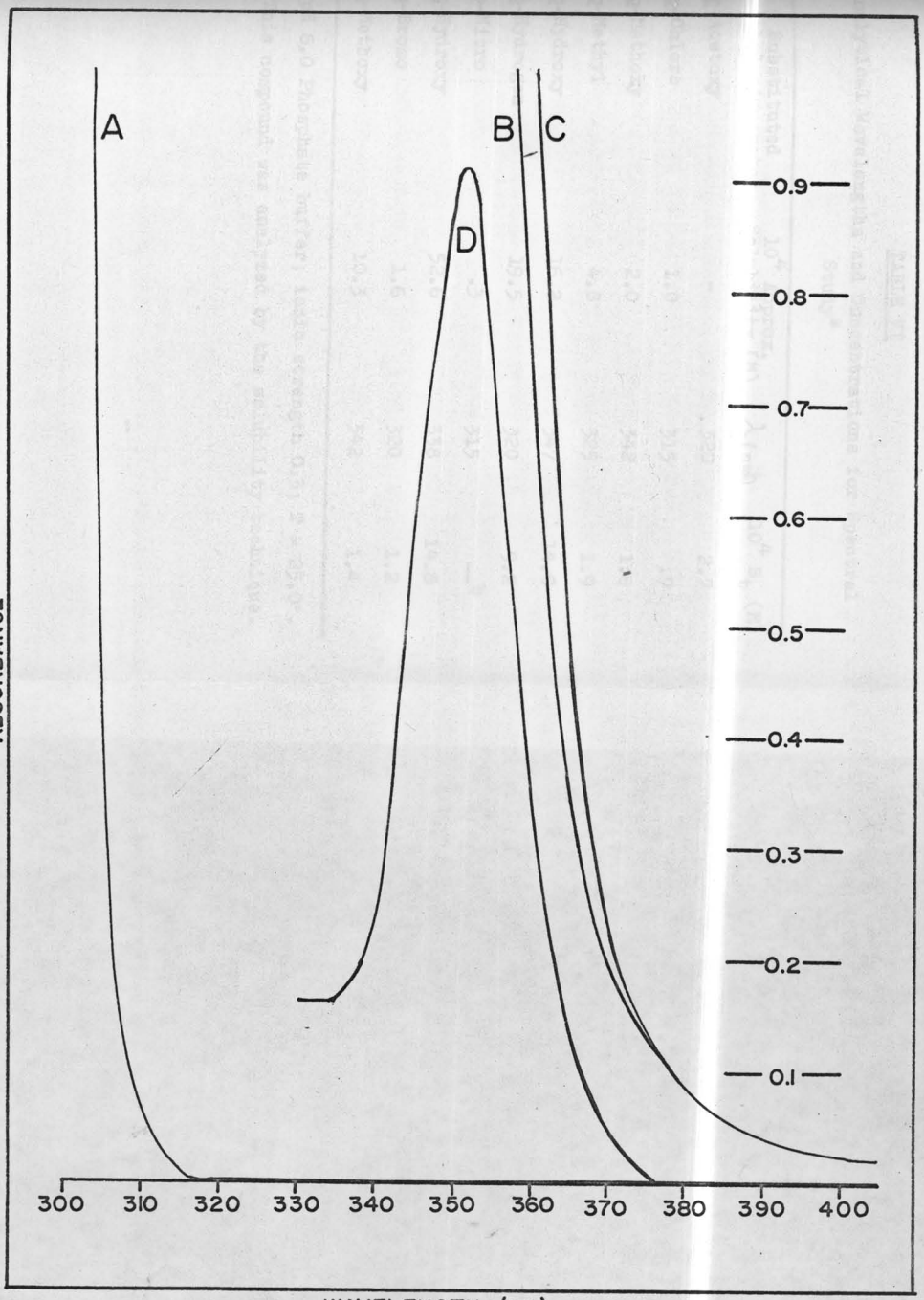

A
B
C
D
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
ABSORBANCE
300
310
320
330
340
350
360
370
380
390
400
WAVELENGTH (nm)

TABLE VI

Analytical Wavelengths and Concentrations for Spectral Study[a]

Substituted methyl cinnamates	10^4 Approx. Solubility (M)	λ (nm)	10^4 S_t (M)
p-Acetoxy	-	320	2.7
p-Chloro	1.0	315	.7
p-Methoxy	2.0	342	1.6
p-Methyl	4.8	325	1.9
p-Hydroxy	16.2	347	14.0
p-Hydrogen	19.5	320	9.2
p-Nitro	.3	315	--[b]
m-Hydroxy	52.6	338	14.8
m-Bromo	1.6	320	1.2
m-Methoxy	10.3	342	1.4

[a]pH 6.0 Phosphate buffer; ionic strength 0.3; T = 25.0°.

[b]This compound was analyzed by the solubility technique.

TABLE VII

Second-Order Rate Constants for Alkaline Hydrolysis of Some Esters

Ester	k_{OH} ($M^{-1}sec^{-1}$)[a] Mean	S.D.[b]	No. of Detns.
o-Acetoxycinnamic acid	1.64	0.07	13
m-Acetoxycinnamic acid	2.33	0.17	15
p-Acetoxycinnamic acid	2.60	0.11	8
Phenyl acetate	1.75	0.02	2
p-Acetylphenyl acetate	5.46	0.03	2
p-Chlorophenyl acetate	2.38	0.03	2
p-Methylphenyl acetate	0.90	0.02	3
o-Nitrophenyl acetate	9.69	0.10	2
p-Nitrophenyl acetate	12.32	0.03	2
Methyl trans-cinnamate	0.08	0.003	10
p-Acetoxybenzalacetone	3.71	0.08	9
Methyl p-acetoxycinnamate[c]	3.90	0.05	9

[a] Experiments were carried out in carbonate or hydroxide buffer; 25.0°; ionic strength 0.3; based on potentiometric measurements of hydroxide activity.

[b] Standard deviation.

[c] k_{OH} is for hydrolysis of the acetoxy group.

1:1 complex stoichiometry with an apparent equilibrium constant K_{11}'; all concentrations are on a molar basis.

a) Solubility method.

$$S_t = \frac{K_{11}' S_o L_t}{1 + K_{11}' S_o} + S_o \qquad \text{(Eq. 1)}$$

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,

$$K_{11}' = \frac{\text{slope}}{\text{intercept}(1-\text{slope})} \qquad \text{(Eq. 2)}$$

b) Spectral method.

$$\frac{b}{\Delta A} = \frac{1}{K_{11} S_t \Delta a (L)} + \frac{1}{S_t \Delta a} \qquad \text{(Eq. 3)}$$

where,

b = cell path length

ΔA = difference in absorbances of substrate in the 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,

$$K_{11}' = \frac{\text{Y-intercept}}{\text{slope}} = -(\text{X-intercept}) \qquad \text{(Eq. 4)}$$

c) Kinetic method.

$$\frac{k_s}{k_s - k_s'} = \frac{1}{q_{11}K_{11}(L)} + \frac{1}{q_{11}} \qquad \text{(Eq. 5)}$$

where,

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

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

q_{11} = fractional decrease in reactivity of the complexed substrate ($q_{11} = 1 - k_{11}/k_s$)

(L) = concentration of uncomplexed ligand.

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

$$K_{11}' = \frac{\text{Y-intercept}}{\text{slope}} = -(\text{X-intercept}) \qquad \text{(Eq. 6)}$$

Upon rearrangement of the equation for the kinetic method we obtain

$$\frac{k_s'}{k_s} = 1 - \frac{q_{11}K_{11}(L)}{1 + K_{11}(L)} \qquad \text{(Eq. 7)}$$

At very low (L), when $K_{11}(L) \ll 1$, this is linear in (L):

$$\frac{k_s'}{k_s} = 1 - q_{11}K_{11}(L) \qquad \text{(Eq. 8)}$$

A criterion for the use of this method is that $K_{11}(L) \leq 0.1$; thus, k_s'/k_s will always be in the range 1.00 to 0.90 and these values should be determinable with the same precision as k_s, i.e., 1-3%. With equation (8), one plots k_s'/k_s *vs.* (L); the slope is $-q_{11}K_{11}$. Or one may rearrange the equation to obtain $k_s' = k_s - k_s q_{11}K_{11}(L)$. A plot of k_s' *vs.* (L) gives a slope of $-q_{11}K_{11}k_s$. Typical plots are shown in Figures 8 and 9. Either plot is suitable and will give the desired $q_{11}K_{11}$; it is preferable, however, to use the first plot, which is in a normalized form and allows immediate comparison between studies at different pH's.

The advantages of this method are: (1) for ligands whose solubility is very low, it allows determination of $q_{11}K_{11}$; (2) it eliminates the need to take reciprocals of very small differences as may arise in the usual kinetic method (Eq. 5); also all values are of equal weight; (3) if the ligand is capable of self-association, then the lower the ligand concentration, the less the association; (4) working at low concentration of ligand

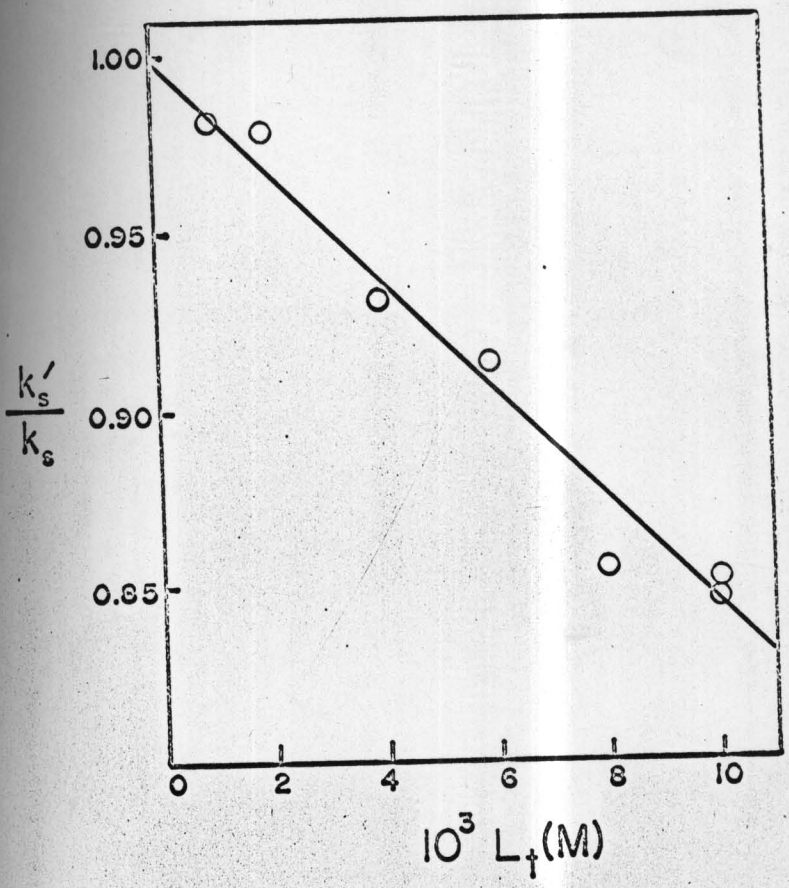


Figure 8. Plot of kinetic data for the methyl cinnamate-theophyllinate system: pH 12.85 hydroxide buffer; 25.0°; ionic strength 0.3; 0.8% CH_3CN.

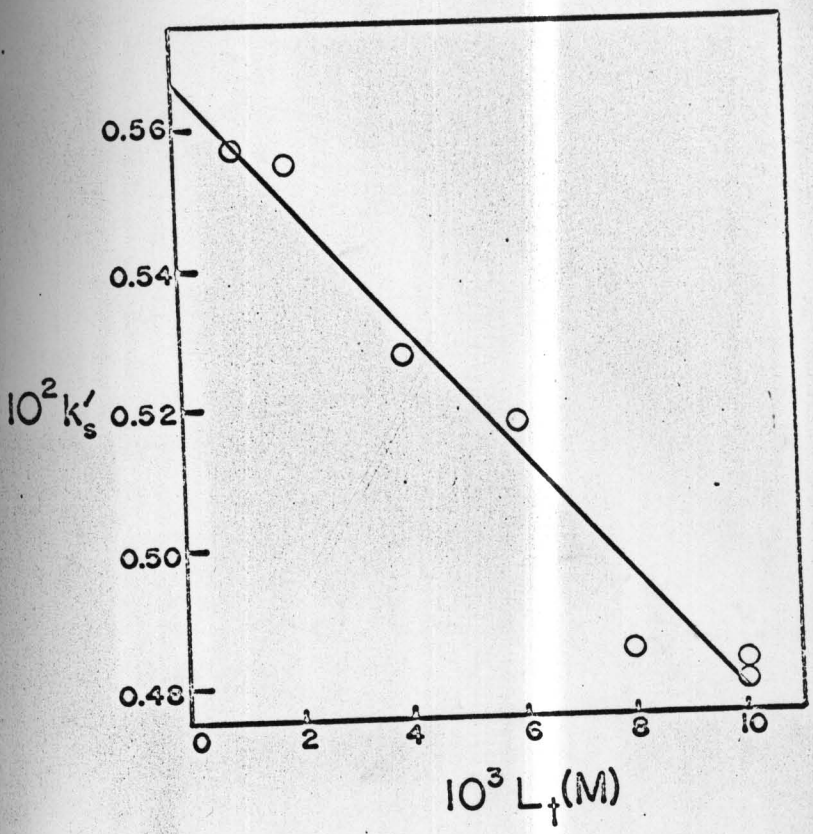


Figure 9. Plot of kinetic data for the methyl cinnamate-theophyllinate system: pH 12.85 hydroxide buffer; 25.00; ionic strength 0.3; 0.8% CH_3CN.

minimizes solvent effects. There are several limitations to the method; (1) the condition $K_{11}(L) \leq 0.1$, limits k_s'/k_s to values between 1.00 and 0.90, which is a small change; (2) the final result ($q_{11}K_{11}$) has about the same precision as in the other method; and (3) it is impossible to separate $q_{11}K_{11}$ into its individual quantities. In conclusion, this method would be utilized when the advantages are greater than for the other method, but we probably would not use this method in preference to the standard method due to the inability to separate q_{11} from K_{11}.

The usual method of least squares was applied to the solubility data, while a weighted least squares analysis was applied to all double reciprocal (kinetic and spectral) plots. These plots were made with the aid of a digital computer* which computed the slope and the Y-intercept. From experience with the experimental system the value of the weighting factor was computed by the formula: $w = d^2/\Sigma d^2$, where d is the denominator of the fraction plotted on the Y-axis (91, 44).

---

*The program for this numerical analysis was written by J. L. Cohen and P. A. Kramer of this laboratory and performed by a Control Data Corporation 1604 digital computer.

RESULTS

A. Complex Reactivity in Relation to Substrate Structure

1. o-, m-, and p-Acetoxycinnamic Acids.

Most of the cinnamate-xanthine complexes previously studied (56b, 44, 33) appear to be unreactive toward hydroxide ion attack. Thus, for these complexes, complex reactivity as determined by the kinetic technique cannot be used as a probe for making structural inferences and consequently it has not been possible to postulate detailed complex structures for these nonisolable complexes. Acetoxycinnamic acid was chosen as a potential substrate on the basis that the acetoxy group may be positioned sufficiently far away from the area of complexation (area of overlap of the ligand) so that nucleophilic attack by hydroxide ion on the complexed structure is not fully inhibited and thus some measure of the complex reactivity will be retained. The acetoxy-cinnamic acids' utility as a probe for complex structure may permit establishment of a limit on the overlap region.

Previous complexation studies on cinnamate anions have shown that coplanarity of the unsaturated side chain with the phenyl ring is an important criterion in determining the extent of complexation (see discussion on page 12 and references therein).

C.P.K. models (92) of acetoxycinnamic acid show that the olefinic side chain is indeed coplanar with the phenyl ring; however, the acetoxy group is almost perpendicular to the remainder of the molecule. It is expected that the acetoxy group is sufficiently far removed from the area of complexation so that it will not disrupt the complex by its lack of coplanarity. Thus we expect the o-, m-, and p-acetoxycinnamic acid anions to behave essentially the same as the cinnamate anion with respect to their complexing tendencies with theophyllinate (2).

Initial kinetic studies on the acetoxycinnamic acids were carried out in phosphate buffer, which showed accelerated rates over those obtained in hydroxide buffer. Since it was assumed that phosphate catalyzes the hydrolysis, all subsequent kinetic studies were carried out in either hydroxide or carbonate buffers.

The rates of alkaline hydrolysis of the acetoxycinnamic acids* in the presence of theophyllinate were determined under pseudo-first order conditions directly in the spectrophotometer cell. Examples of the kinetic

---

*Some substituted cinnamic acids have been shown to undergo pH-dependent decarboxylation. The half-life of p-hydroxycinnamic acid at 100°C, and pH 11.8, was found to be about 173 hours (93); hence, the decarboxylation of the hydrolysis product, p-hydroxycinnamic acid, does not occur to a significant extent at 25°C during the length of time we followed ester hydrolysis.

reciprocal plots of the apparent second-order rate constants for p-acetoxycinnamic acid and o-acetoxycinnamic acid are given in Figures 10 and 11, respectively. The K_{11}' calculated from a cumulative plot is 7.5 M^{-1} for the p-acetoxycinnamic acid anion-theophyllinate system and 14 M^{-1} for the o-acetoxycinnamic acid anion-theophyllinate system. The experimental uncertainty is about 10-15%. For the m-acetoxycinnamic acid anion, the experimental uncertainty was greater (25-30%); a cumulative K_{11}' of 10.5 M^{-1} was obtained.

From the intercept of the kinetic reciprocal plot we can compute the value of q_{11}, the fractional decrease in reactivity of the complexed substrate. It is defined mathematically,

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

where k_{11} is the rate constant for the reaction of complex with the reagent (in this case, hydroxide ion), and k_s is the rate constant for the reaction of substrate with the reagent. The respective values of q_{11} (1/Y-intercept) for the o-, m-, and p-acetoxycinnamic acids are 0.41, 0.44, and 0.42; within the accuracy of the data, we conclude that these are all 0.4. This q_{11} value suggests that the complexed ester retains about 60% of its reactivity towards hydroxide attack. The low q_{11} values obtained also suggest that complexing is not

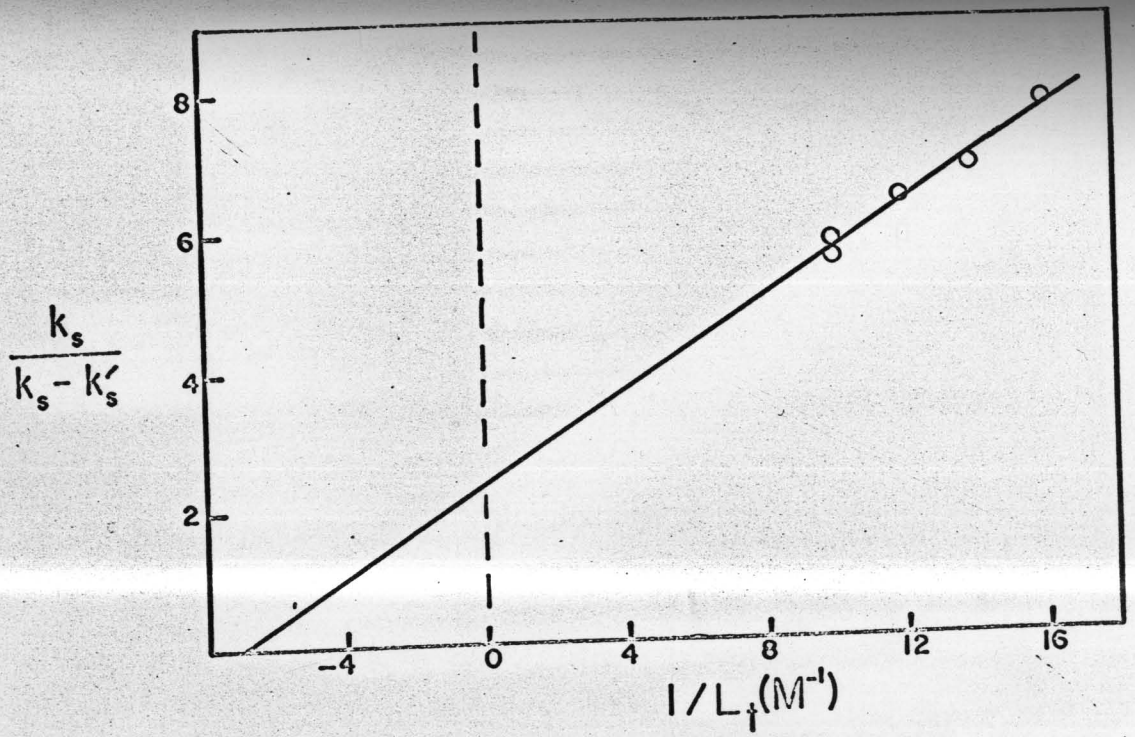


Figure 10. Plot of kinetic data for the p-acetoxycinnamic acid-theophyllinate system: pH 11.80; ionic strength 0.3; 1.6% CH_3CN; 25.0°.

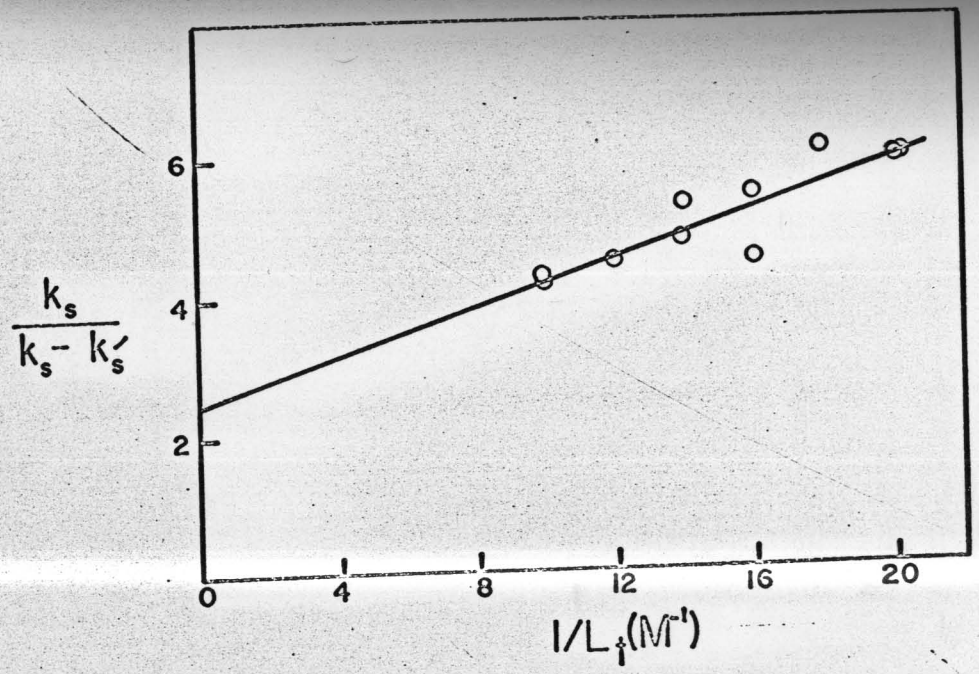


Figure 11. Plot of kinetic data for the o-acetoxycinnamic acid-theophyllinate system: pH 11.95; ionic strength 0.3; 1.6% CH_3CN; 25.0°.

due to a direct interaction of the ligand with the acetoxy group and that, in fact, the o-, m-, and p-positions must be essentially free of steric hindrance from the ligand.

A significant catalysis of the hydrolysis of p-nitrophenyl acetate by theophylline anion was observed by Kramer (94). This caused some concern as the acetoxycinnamic acids are themselves monosubstituted phenyl acetates (the substituent being -CH=CH-COOH) and the low q_{11} values which were obtained could have been a composite effect of high q_{11} values plus some concurrent catalysis. Since we were interested in making an estimate of the catalytic rate constant for the acetoxycinnamic acids to obtain corrected K_{11} and q_{11} values, we chose some other monosubstituted phenyl acetates as reasonable models. The nucleophilic reactivity of various bases towards substituted phenyl acetates has already been studied as a function of the pK_a of the leaving group (95, 96, 22); therefore we tried to correlate our data with the pK_a of the leaving group.

The apparent second-order rate constants for the hydrolysis of the various phenyl acetates in the absence and presence of 0.1 M theophylline anion are given in Table VIII, along with the pK_a of the parent phenols. Figure 12 demonstrates the relationship between the k_{OH} and the pK_a of the leaving group, showing that the

TABLE VIII

Apparent Second-Order Rate Constants of Some Esters in the Absence and Presence of 0.1 M Theophylline Anions[a]

Compound	k_{OH}	log k_{OH}	k_s'	pK^b
p-Nitrophenyl acetate	12.32	1.10	45.10	7.10
o-Nitrophenyl acetate	9.68	0.99	46.90	7.23
p-Acetylphenyl acetate	5.45	0.74	9.68	8.10
p-Chlorophenyl acetate	2.38	0.38	1.77	9.40
p-Acetoxycinnamic acid	2.60	0.42	2.11	9.41
m-Acetoxycinnamic acid	2.33	0.37	1.82	9.96
o-Acetoxycinnamic acid	1.64	0.22	1.20	9.89
Phenyl acetate	1.75	0.24	--	9.95
p-Methylphenyl acetate	0.90	-0.05	0.50	10.25

[a] pH 11.8 hydroxide buffer; ionic strength 0.3; 1.6% CH_3CN; T = 25.0°; rate constants in M^{-1} sec^{-1}.

[b] pK of the parent phenols (97). The hydroxycinnamic acid pK's were determined spectrophotometrically at 25°. The pK_1's for these compounds are: o- = 4.46; m- = 4.34; and p- = 4.35.

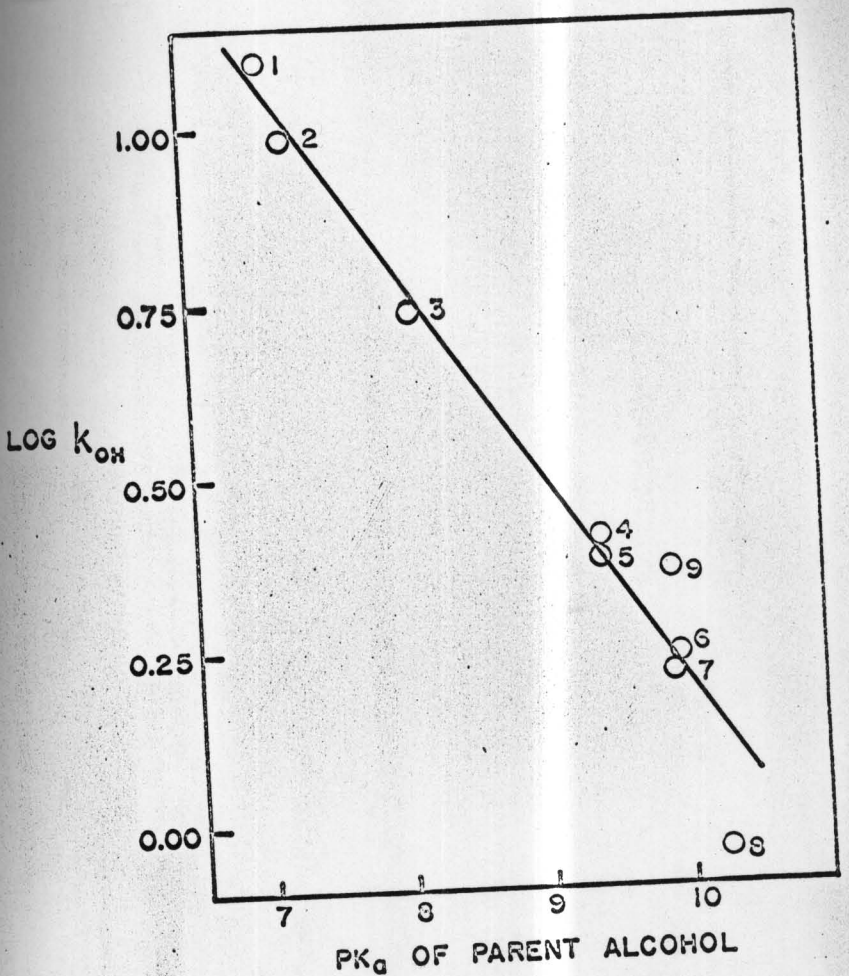


Figure 12. Apparent second-order rate constants of some substituted phenyl acetates as a function of the pK_a of the leaving group, pH 10.50; ionic strength 0.3; 1.6% CH_3CN; 25.0°. 1) p-nitrophenyl acetate; 2) o-nitrophenyl acetate; 3) p-acetylphenyl acetate; 4) p-acetoxycinnamic acid anion; 5 p-chlorophenyl acetate; 6) phenyl acetate; 7) o-acetoxycinnamic acid anion; 8) p-methylphenyl acetate; 9) m-acetoxycinnamic acid anion.

substrates and reactions are well-behaved. The plot of log (k_s'/k_{OH}) with ligand concentration of 0.1 M theophylline *vs.* pK_a, Figure 13, shows a decrease in relative rate with an increase in the pK_a of the leaving group. Inhibition is seen for all substrates with a pK_a greater than 9; hence, we concluded that theophyllinate is not significantly catalyzing the hydrolysis of the acetoxycinnamic acid anions, since the phenolic pK_a's of these parent phenols are 9.4-9.9 (Table VIII).

An unanticipated associated problem was revealed by these data: Why should we observe any inhibition for the phenyl acetates without a cinnamate structure? To answer this question, a kinetic study of *p*-chlorophenyl acetate with theophylline anion was performed; the results are shown in Figure 14. Values of K_{11}' of 4.7 M^{-1} and $q_{11} = 1$ were obtained. Hence the phenyl acetates appear to complex to some extent with theophyllinate. Theophyllinate also complexes with the acetoxycinnamic acid anion; however, the relative position of substrate to ligand in the complex must be different in the two molecules because we obtained a q_{11} of 1 for *p*-chlorophenyl acetate, indicating a nearly complete loss in reactivity of the complex, while we obtained a q_{11} of 0.4 for the acetoxycinnamic acid anion.

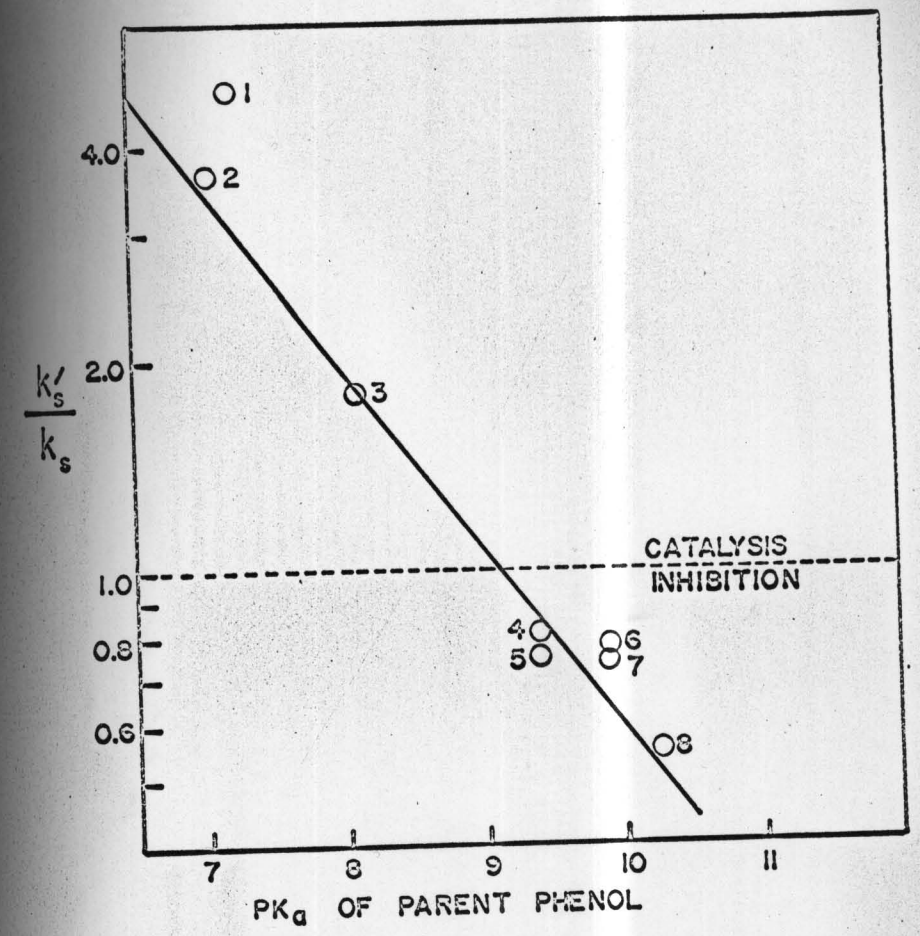


Figure 13. The relative rate of hydrolysis of some substituted phenyl acetates in the absence and presence of 0.1 M theophyllinate as a function of the pK_a of the leaving group, pH 10.50; ionic strength 0.3; 1.6% CH_3CN; 25.0°. 1) p-nitrophenyl acetate; 2) o-nitrophenyl acetate; 3) p-acetylphenyl acetate; 4 p-acetoxycinnamic acid anion; 6) m-acetoxycinnamic acid anion; 7) o-acetoxycinnamic acid anion; 8) p-methylphenyl acetate.

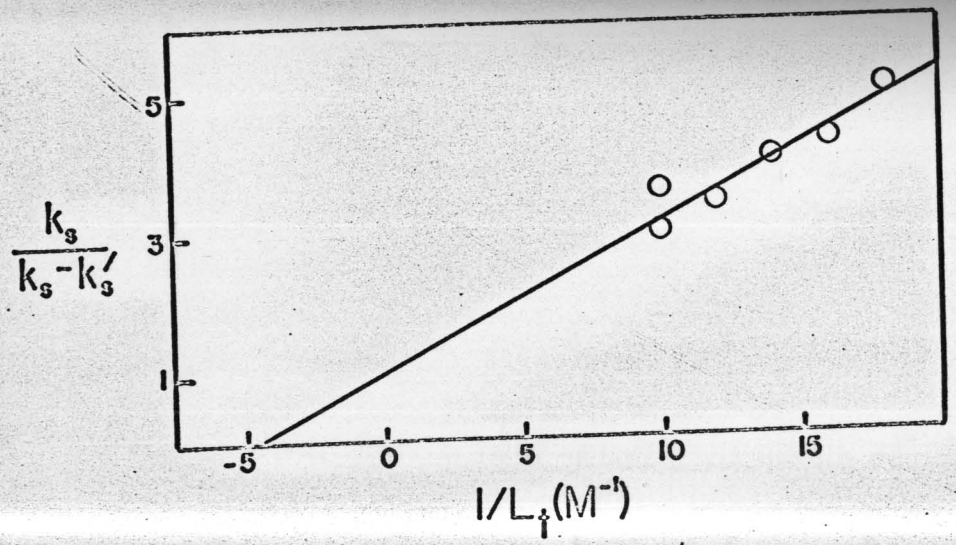


Figure 14. Plot of kinetic data for the p-chlorophenyl acetate-theophyllinate system: pH 11.70; ionic strength 0.3; 1.6% CH_3CN; 25.0°.

2. Methyl p-Acetoxycinnamate and p-Acetoxybenzalacetone.

By analogy with the cinnamic acid anion-theophylline, $K_{11}' = 12\ M^{-1}$, and methyl cinnamate-theophylline, $K_{11}' = 25\ M^{-1}$, systems (33), we felt that by removing the negative charge from the acetoxycinnamic acid anion (see structure 2) we might increase K_{11} without appreciably affecting q_{11}. Kinetic studies of p-acetoxybenzalacetone with theophyllinate gave a K_{11}' of $7\ M^{-1}$ with a q_{11} of 0.4 and methyl p-acetoxycinnamate with theophyllinate gave a K_{11}' of $8\ M^{-1}$ with a q_{11} of 0.5. The change in substrate to a neutral molecule did not significantly alter either q_{11} or K_{11}.

A spectral study of the methyl p-acetoxycinnamate-theophylline system was carried out and yielded a K_{11}' value of $14\ M^{-1}$. This result is not unexpected as previous studies gave larger stability constants with theophylline than with theophyllinate (56b, 3).

Because larger values of K_{11} were not obtained with these substrates, and hence we were unable to increase the reliability of the inhibition measurements, we decided to screen some other substituted xanthines in an attempt to locate a better system for study (see Table IX). The maximum inhibitions observed for the substituted xanthines were not significantly greater than those for the theophylline anion, even when the xanthine

TABLE IX

Change in Rate for the Acetoxycinnamic Acids and p-Acetoxybenzalacetone with Various Ligands[a]

Ligand	Ligand Molarity	p-Acetoxy-cinnamic acid	m-Acetoxy-cinnamic acid	o-Acetoxy-cinnamic acid	p-Acetoxy-benzalacetone
Theophylline anion	0.100	--	--	--	--
Caffeine	0.079	--	--	--	--
Theophylline 7-acetic acid	0.068	0	--	--	--
8-Chlorotheophylline anion	0.071	--	--	--	--
8-Bromotheophylline anion	0.039	--	0	--	--
8-Iodotheophylline anion	0.066	--	+	--	--
7-(2-Chloroethyl)-theophylline	0.023	--	+	--	--
6-Methoxycaffeine	0.009	0	-	0	-
Propylene glycol	0.200	+	+	+	+
7-(2,3-Dihydroxypropyl)-theophylline	0.206	+++	+++++	++++	+++++

[a]Each - or + sign signifies about a 10% change; 0 signifies no change; pH 10.8 carbonate buffer; ionic strength 0.3; 1.6% CH_3CN; T = 25.0°.

was uncharged. One curious result did emerge, however; a relatively large catalysis was observed for the substrates when the ligand was 7-(2,3-dihydroxypropyl)-theophylline. This effect was investigated and the results are described in the next section.

B. Complex Reactivity in Relation to Ligand Structure

1. 7-(2,3-Dihydroxypropyl)-theophylline.

As shown in Table IX, an unexpected catalysis was observed with 7-(2,3-dihydroxypropyl)-theophylline and the o-, m-, and p-acetoxycinnamic acid anions and p-acetoxybenzalacetone. This catalysis was explored with p-acetoxybenzalacetone to see whether the catalysis occurred via complexation or by direct nucleophilic attack by the ligand on the substrate. A plot of the relative rates for the alkaline hydrolysis of p-acetoxybenzalacetone with this ligand is shown in Figure 15. Spectral studies were carried out on methyl p-acetoxycinnamate, p-acetoxybenzalacetone, and on p-acetoxycinnamic acid; the data are given in Table XIII.

2. Related ligands.

After this initial experimentation, the investigation took two directions: (1) variations in the ligand to determine what component(s) of the 7-(2,3-dihydroxypropyl)-theophylline structure were

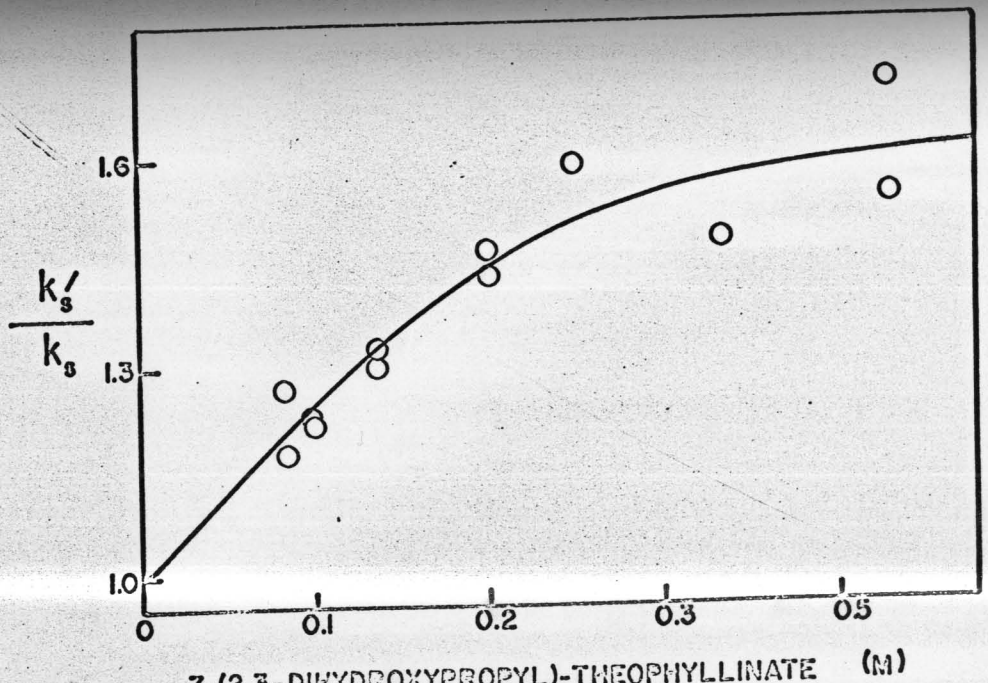


Figure 15. Relative rates for the alkaline hydrolysis of p-acetoxybenzalacetone at various concentrations of 7-(2,3-dihydroxypropyl)-theophylline: pH 10.80; ionic strength 0.3; 1.6% CH_3CN; 25.0°.

required for catalysis, and (2) variations in the ring position (o, m, and p) of a substituent on the substrate to find out if this has a significant effect on the catalysis. We hoped that the combined results from (1) and (2) would determine if the catalysis occurred through complex formation or by simple nucleophilic attack. Therefore, the methyl o-, m- and p-acetoxy-cinnamates were chosen as substrates. See Table V for structures. It was expected that the K_{11} values would be similar to previously obtained data. A one-point screening study was carried out with these substituted methyl cinnamates and various xanthines as ligands. The results of this screening are given in Table X.

After inspection of the data, we concluded that the result with theophyllinate is definitely anomalous, at least with the ortho and meta compounds. Therefore, we decided to neglect the theophyllinate systems in trying to account for the rest of the results. It was also decided to neglect caffeine mainly on the basis of its limited solubility.

Since we are concerned with determining the component(s) of the 7-(2,3-dihydroxypropyl)-theophylline molecule responsible for the catalysis, 7-(n-propyl)-theophylline was chosen as the reference ligand. Table XI-A was constructed to show the differences in

TABLE X

Percentage Change in Rate of the Methyl Acetoxycinnamate Hydrolysis with Various Ligands[a]

Ligand	Methyl *p*-acetoxy cinnamate	Methyl *m*-acetoxy cinnamate	Methyl *o*-acetoxy cinnamate
Theophyllinate	-25	-15	+60
Caffeine	-25	-22	- 9
7-(*n*-Propyl)-theophyllinate	-40	-47	-37
7-(2-Hydroxypropyl)-theophyllinate	-22	-10	+ 6
7-(3-Hydroxypropyl)-theophyllinate	-25	-25	-12
7-(2,3-Dihydroxypropyl)-theophyllinate	+40	+37	+46
Propylene glycol	+ 8	+10	+10

[a]pH 10.8 carbonate buffer; ionic strength 0.3; 1.6% CH_3CN; T = 25.0°; all ligands are 0.2 *M* except as noted: 0.1 *M* theophyllinate; 0.073 *M* caffeine.

percentage change for a common substrate with different ligands. Similar operations can be performed for common ligands with different substrates, and these results are in Table XI-B. The magnitude of the differences are such that, within the limits of this study, we concluded that the behavior of all the substrates are similar and chose the methyl p-acetoxycinnamate to investigate more thoroughly.

In order to facilitate handling of the catalytic data, we have utilized the equation

$$k_s' - k_s = f_{11}(k_{11} - k_s) \qquad \text{(Eq. 9)}$$

where the k's have the usual meaning, recognizing $k_s' > k_s$ and $k_{11} > k_s$ for this system. f_{11} is the fraction of the substrate present as complex (56a) or

$$f_{11} = \frac{K_{11}(L)}{1 + K_{11}(L)} \qquad \text{(Eq. 10)}$$

and combining equations (9) and (10) and inverting gives

$$\frac{1}{k_s' - k_s} = \frac{1}{(k_{11} - k_s)K_{11}(L)} + \frac{1}{(k_{11} - k_s)} \qquad \text{(Eq. 11)}$$

Multiplying this equation through by k_s, rearranging, and introducing the definition

TABLE XI

A. Differences in % Change of Rate of Hydrolysis of a Common Methyl Acetoxycinnamate with Different Ligands

Ligand compound*	Substrate o	m	p
2,3HOPr-nPr	+83	+84	+80
2HOPr-nPr	+15	+22	+18
3HOPr-nPr	+46	+37	+15

B. Differences in % Change of Rate of Hydrolysis of a Common Ligand with Various Substrates

Substrate compound	Ligand	Net % difference
o - p	2,3HOPr	+ 1
	2HOPr	+10
	3HOPr	+34
	nPr	+ 3
o - m	2,3HOPr	+ 9
	2HOPr	+13
	3HOPr	+19
	nPr	+10

*For convenience, symbols will be assigned to the ligands: 7-(n-propyl)-theophylline = nPr; 7-(3-hydroxypropyl)-theophylline = 3HOPr; 7-(2-hydroxypropyl)-theophylline = 2HOPr; 7-(2,3-dihydroxypropyl)-theophylline = 2,3HOPr.

$$p_{11} = \frac{k_{11}}{k_s} - 1 \qquad \text{(Eq. 12)}$$

we obtain the normalized form of the equation

$$\frac{k_s}{k_s' - k_s} = \frac{1}{p_{11}K_{11}(L)} + \frac{1}{p_{11}} \qquad \text{(Eq. 13)}$$

In a system in which catalysis occurs upon complexing, p_{11} can take any positive value. K_{11} and p_{11} are, of course, experimentally determined in the usual manner. A typical catalysis plot is seen in Figure 16.

The relative rate of hydrolysis of methyl p-acetoxy-cinnamate in the presence of 7-(n-propyl)-theophylline is seen in Figure 17. Some data used to determine apparent 1:1 complexation equilibrium constants are shown in Table XII and Figures 18-20 and a summary of the constants is presented in Table XIII. Discussion of the results from this section will be presented more fully later.

C. Structure-Stability Relationships in Substituted Methyl Cinnamates.

A powerful approach to the determination of reaction mechanism is the utilization of structural variations in the reactants, followed by observation of the effects of these structural changes on the experimental quantity measured, in our case equilibrium or rate constants. This approach has been applied to complex formation between xanthines and cinnamate esters (33). Structural

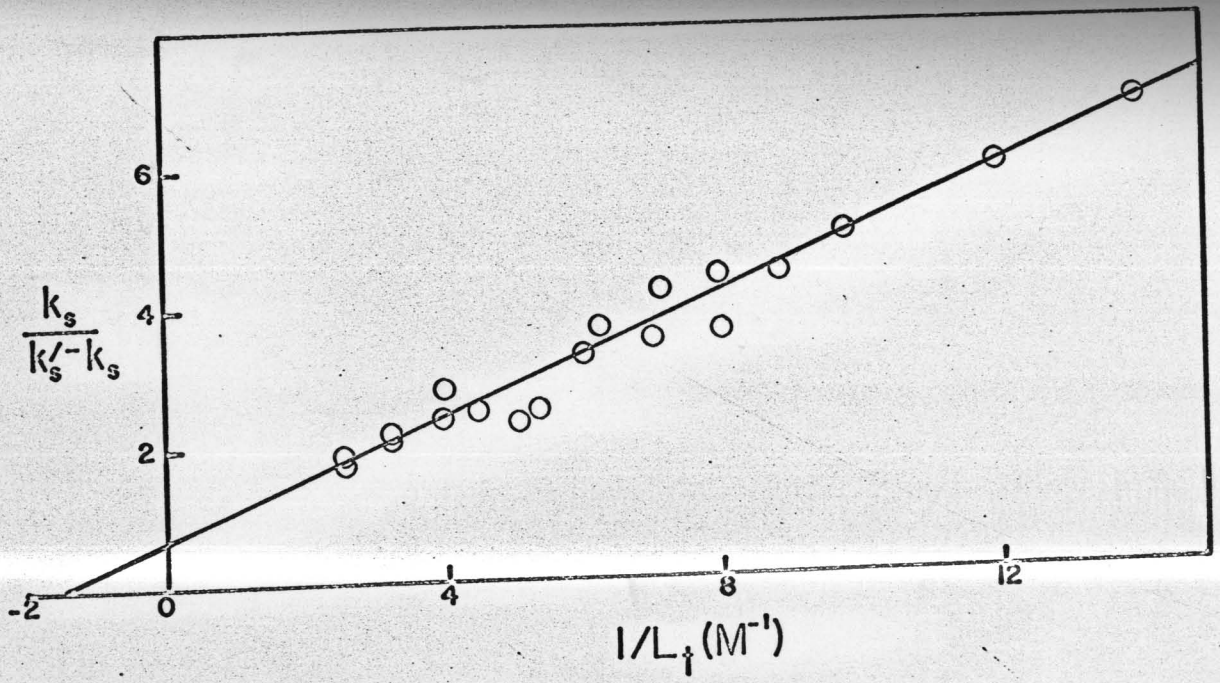


Figure 16. Plot of kinetic data according to equation (13) for the methyl p-acetoxycinnamate-7-(2,3-dihydroxypropyl)-theophyllinate system: pH 10.89; ionic strength 0.3; 25.0°.

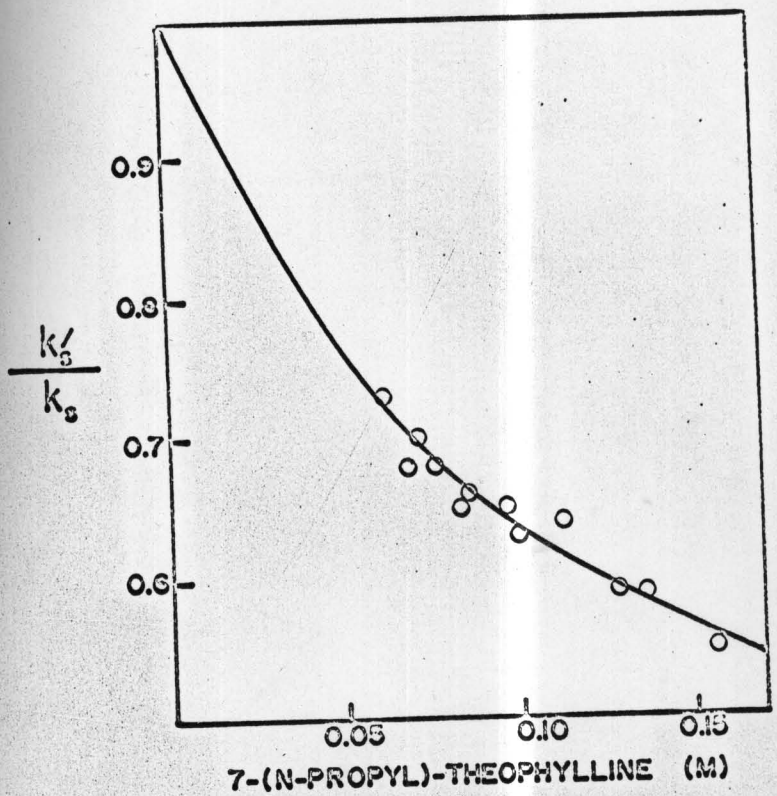


Figure 17. Relative rate of hydrolysis of methyl p-acetoxycinnamate in the presence of 7-(n-propyl)-theophylline (data from Table XII).

TABLE XII

Apparent Second-Order Rate Constants for the Alkaline Hydrolysis of Methyl p-Acetoxycinnamate in the Presence of Varying Concentrations of 7-(n-Propyl)-theophyllinate[a]

7-(n-Propyl)-theophylline (M)	k_{OH} ($M^{-1}sec^{-1}$)	k_s'/k_s
.0000	3.72	
.0625	2.72	.73
.0677	2.54	.68
.0714	2.60	.70
.0758	2.53	.68
.0833	2.42	.65
.0853	2.44	.66
.0961	2.42	.65
.1000	2.34	.63
.1124	2.32	.64
.1282	2.22	.59
.1354	2.20	.59
.1563	2.05	.55

[a]25.0°; 1.6% CH_3CN; ionic strength 0.3; composite of two studies at pH 10.84 and pH 11.23.

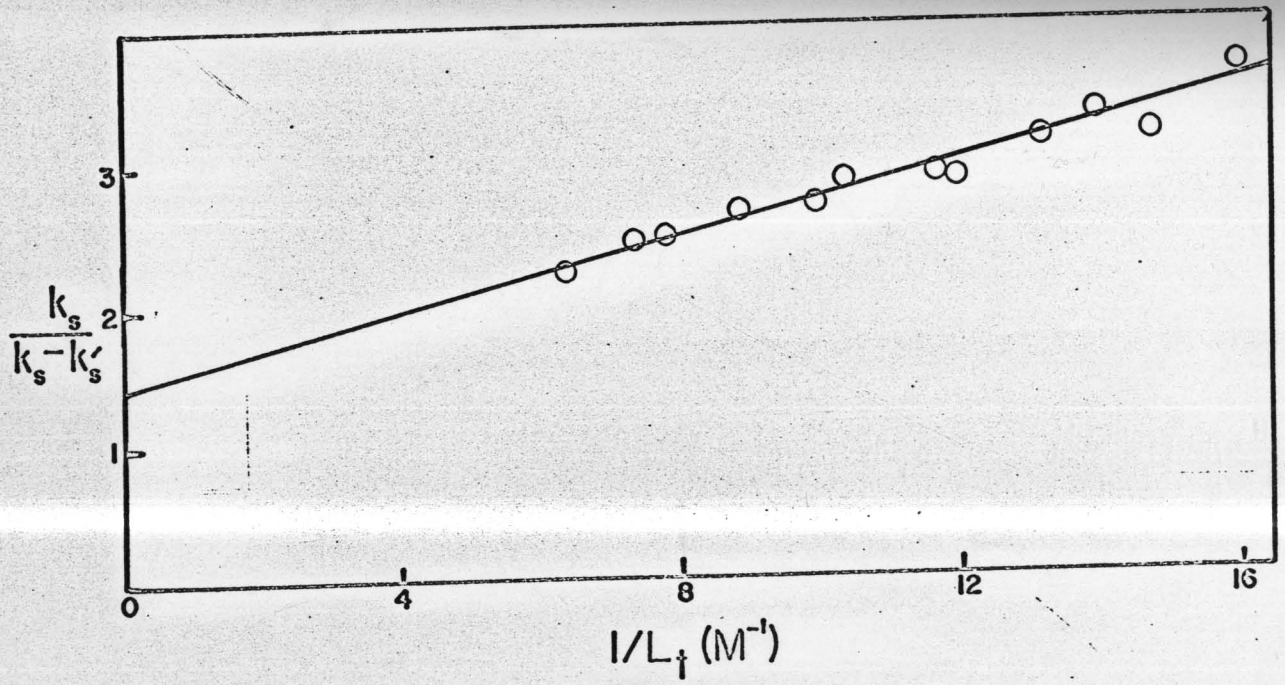


Figure 18. Plot of kinetic data for the methyl p-acetoxycinnamate-7-(n-propyl)-theophyllinate system: pH 10.84; ionic strength 0.3; 25.0°.

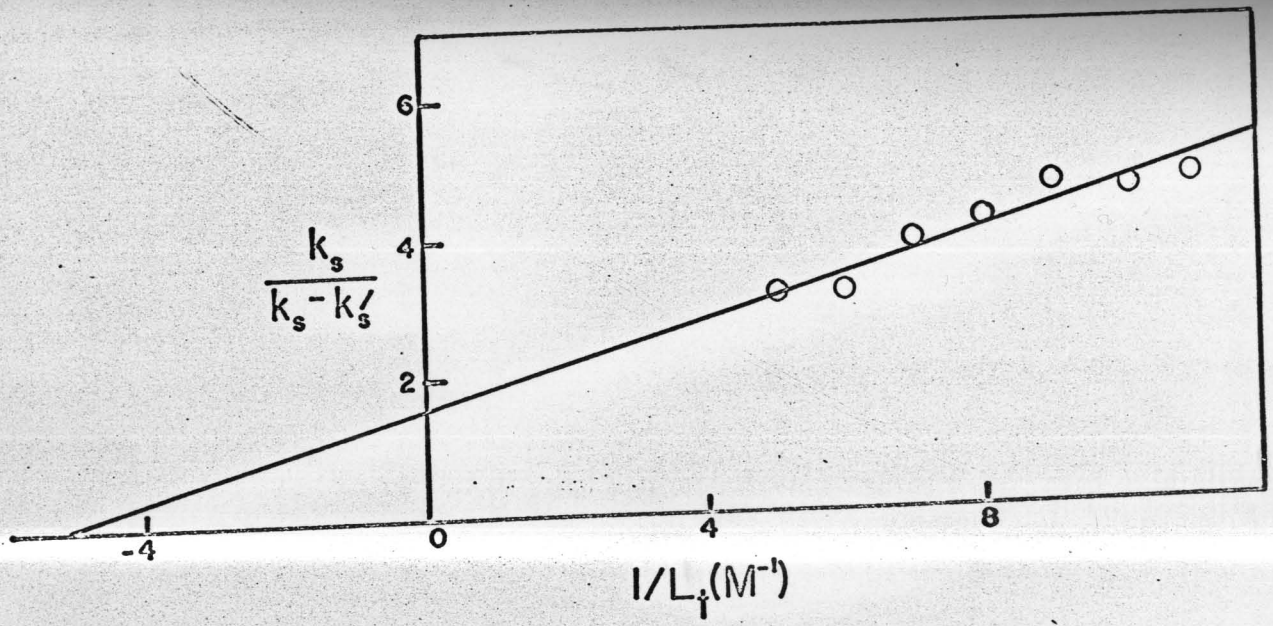


Figure 19. Plot of kinetic data for the methyl p-acetoxycinnamate-7-(2-hydroxypropyl)-theophyllinate system: pH 10.86; ionic strength 0.3; 25.0°.

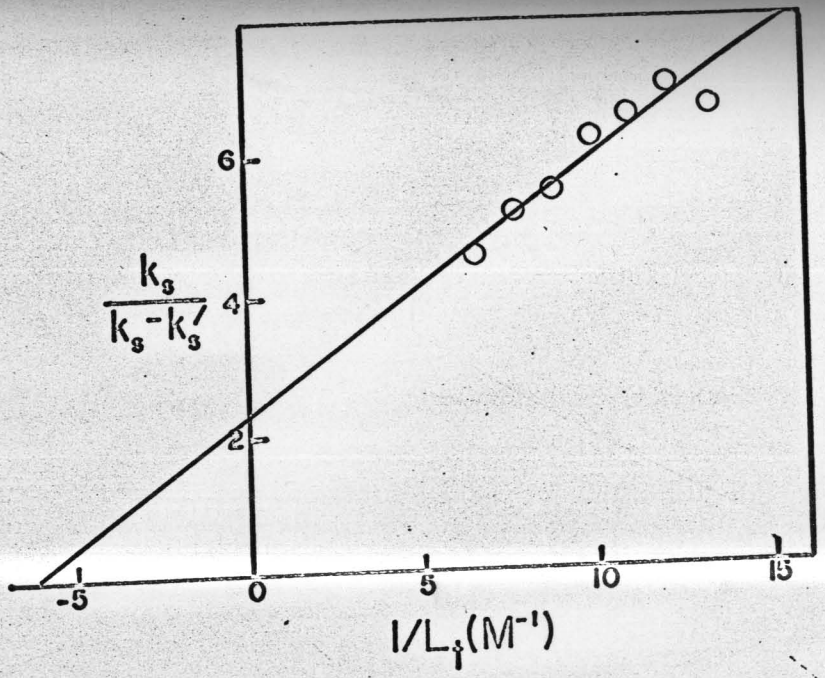


Figure 20. Plot of kinetic data for the methyl p-acetoxycinnamate-7-(3-hydroxypropyl)-theophyllinate system: pH 10.86; ionic strength 0.3; 25.0°.

TABLE XIII

Complexing Data for Some Substituted Xanthines

Ligand	Substrate	Spectral $K_{11}'(M^{-1})$	Kinetic $K_{11}'(M^{-1})$	Kinetic q_{11}
7-(2,3-Dihydroxypropyl)-theophylline	Methyl p-acetoxycinnamate	11	2	---
7-(2,3-Dihydroxypropyl)-theophylline	p-Acetoxybenzalacetone	6	2	---
7-(2,3-Dihydroxypropyl)-theophylline	p-Acetoxycinnamic acids	4		
7-(n-Propyl)-theophylline	Methyl p-acetoxycinnamate	20	10	0.7
7-(2-Hydroxypropyl)-theophylline	Methyl p-acetoxycinnamate		5	0.6
7-(3-Hydroxypropyl)-theophylline	Methyl p-acetoxycinnamate		5	0.4
Theophylline	Methyl p-acetoxycinnamate	14		
Theophylline anion	p-Acetoxybenzalacetone		7	0.4
Theophylline anion	p-Acetoxycinnamic acid anion		7.5	0.4
Theophylline anion	m-Acetoxycinnamic acid anion		11	0.4
Theophylline anion	o-Acetoxycinnamic acid anion		14	0.4

variations both in the acyl portion of the carboxylic acid ester (R in $R-COOCH_3$) and in the alkyl portion of the ester (R in $C_6H_5-CH=CH-COOR$) have already been studied (33); however, no orderly study had heretofore been undertaken to study the effect of substituents at various positions on the phenyl ring of methyl cinnamate.

We chose a series of meta and para substituents, guided by Hammett's sigma values, to cover the entire range of electronic effects, from the electron-donating methoxy and hydroxy groups to the very electron-withdrawing nitro group. We were interested in whether the complex stability constants might: (1) vary in a well-behaved order with the change in electronic properties of the substituents; or (2) display a change suggestive of a change in mechanism. It was desirable that the ligand interact with an apparent 1:1 stoichiometry, not forming multiple complexes; hence, theophylline was the ligand selected for this study (62). The solubilities of the substituted methyl cinnamates and the analytical wavelengths chosen for the spectral studies are given in Table VI.

Most of the compounds chosen exhibited light sensitivity; this was evidenced by a decrease in absorbance with time both in aqueous and organic solvents. This phenomenon was first observed with methyl p-nitrocinnamate and later found for the other substrates,

except for methyl cinnamate itself. Thus, preparation of the stock solution of ester was followed immediately by dilution and equilibration at 25.0°. About 45 minutes was required for a complete spectral study.

With methyl p-nitrocinnamate the magnitude of the absorbance change caused by the spectral shift was too small to be measured accurately (36); hence the stability constant was obtained by solubility analysis (this was carried out in brown vials to protect the substrate from light). The spectral technique of determining the analytical wavelength and measuring the change in absorbance directly (discussed in the experimental section) was utilized for the remainder of the substrates. Direct measurements of the difference spectrum allowed us to read the change in absorbance at the maximum change for methyl m- and p-hydroxycinnamate and for methyl m- and p-methoxycinnamate. Thus, for these substrates and for methyl p-methylcinnamate no correction was necessary for the absorbance of theophylline. Representative data are given in Table XIV and Figures 21-23 with a summary of all the data given in Table XV. A simple statistical analysis of the substituted methyl cinnamate data yielded a mean K_{11}' of 22 M^{-1} with a standard deviation of only 4.6. Thus we can conclude that there are no significant differences in K_{11}' over this wide range of structural alteration.

TABLE XIV

Spectral Analysis Data for Substituted Methyl Cinnamates with Varying Concentrations of Theophylline[a]

L_t (M)	ΔA for Substituted Methyl Cinnamates[b]			
	m-MeO	p-Cl	m-Br	p-Me
33.34	.538	.583	--	1.050
38.78	.494	.503	.340	.959
43.88	.453	.477	.320	.890
49.04	.417	.450	.286	.818
53.79	.388	.417	.275	.777
59.56	.380	.382	.249	.730

[a] pH 6.0 phosphate buffer; ionic strength 0.3; 1% CH_3CN; 25.0°.

[b] m-MeO, p-Cl, and p-Me read in 5 cm cells; m-Br read in 10 cm cells.

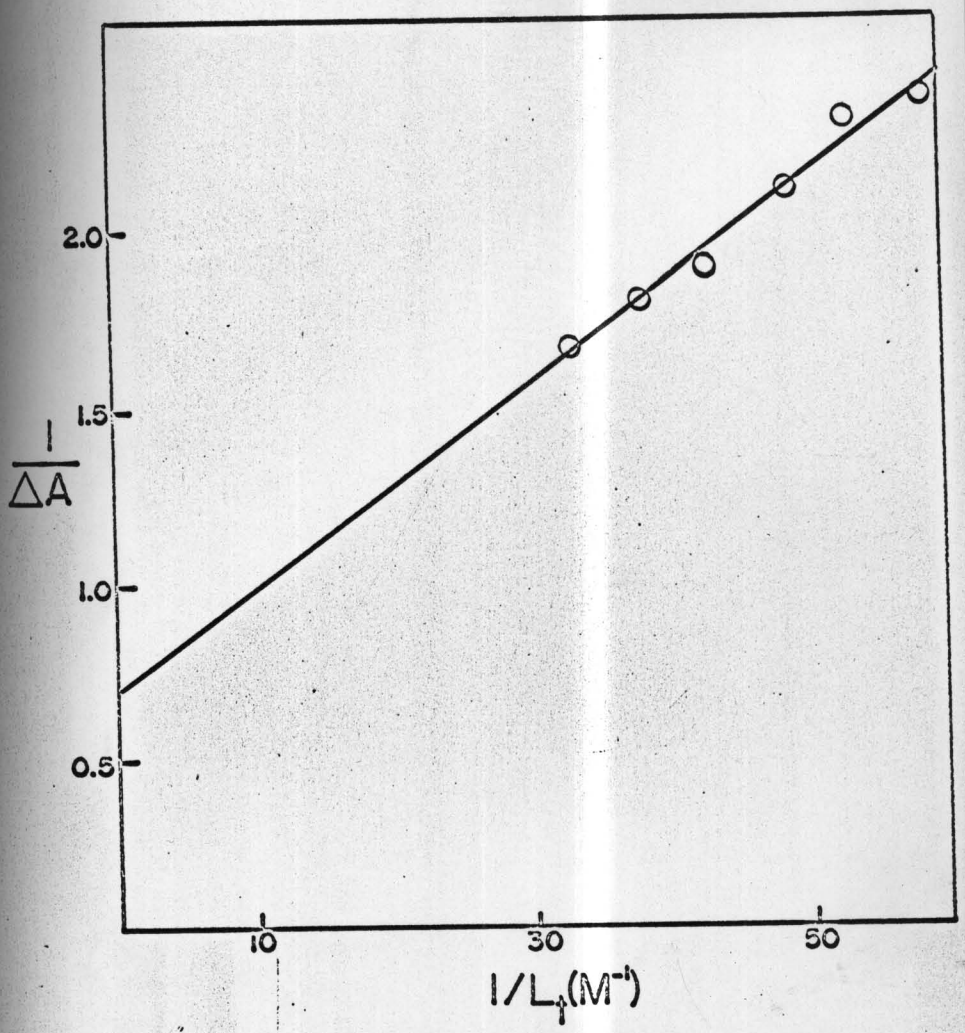


Figure 21. Plot of spectral data for methyl p-hydroxycinnamate-theophylline system: pH 6.0 phosphate buffer; ionic strength 0.3; 1% CH_3CN; 25.0°.

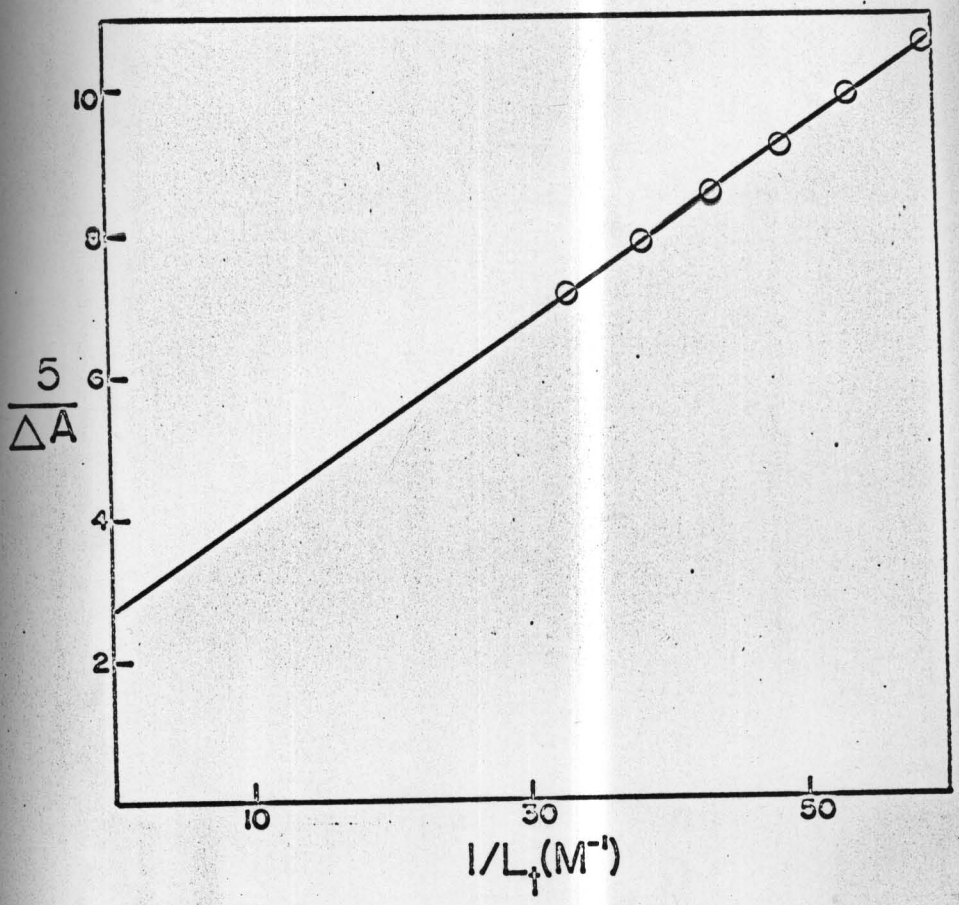


Figure 22. Plot of spectral data for methyl p-methoxycinnamate-theophylline system: pH 6.0 phosphate buffer; ionic strength 0.3; 1% CH_3CN; 25.00°.

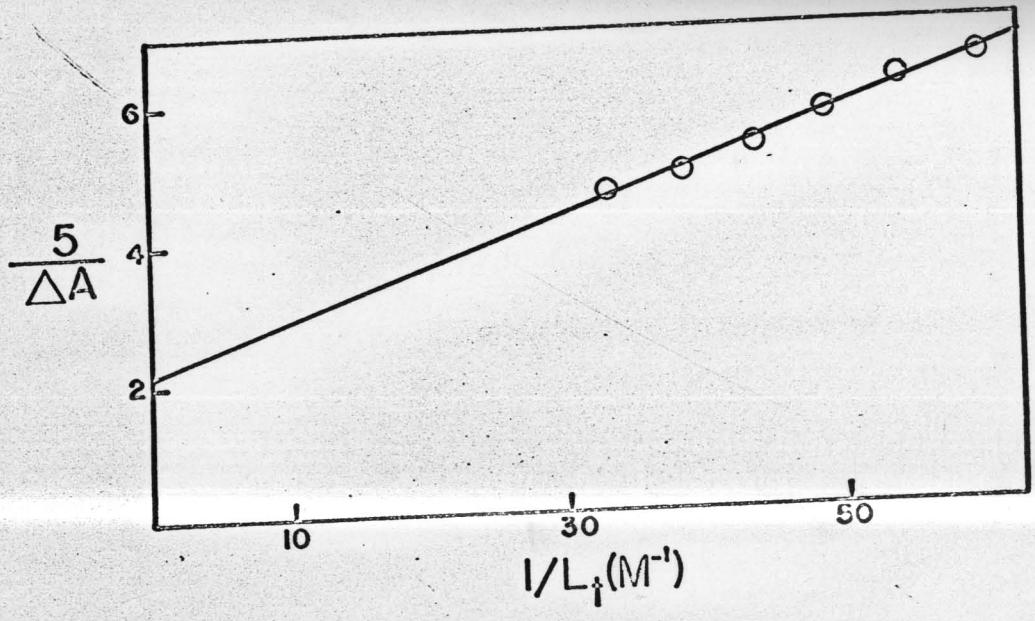


Figure 23. Plot of spectral data for methyl p-methyl cinnamate-theophylline system: pH 6.0 phosphate buffer; ionic strength 0.3; 1% CH_3CN; 25.0°.

TABLE XV

Apparent Stability Constants of Some Substituted Methyl Cinnamates with Theophylline[a]

Substituted Methyl cinnamates	Hammett's σ[b]	$K_{11}'(M^{-1})$[c]
p-Hydroxy	-0.37	22
p-Methoxy	-0.27	19
p-Methyl	-0.17	30
p-Hydrogen	0.00	25
m-Hydroxy	0.12	17
m-Methoxy	0.12	25
p-Chloro	0.23	20
p-Acetoxy	0.31	14
m-Bromo	0.39	21
p-Nitro	0.78	25

[a] p-Nitro determined by solubility analysis; all others by spectral analysis; pH 6.00 phosphate buffer; ionic strength 0.3; 1% CH_3CN; 25.0°.

[b] Reference 98.

[c] Average value of 2-3 determinations at different substrate concentrations.

D. Medium Effects on Some Neutral Complexes

The literature contains scattered reports of studies on medium effects on molecular complexes. Davis and Symons studied the iodine-naphthalene complex (99) and Emslie and Foster studied the tetrachlorophthalic anhydride-hexamethylbenzene complex (100) in various organic solvents. In both cases the observed changes in complex stability appear to be due to an interaction of the solvent with the donor. These and many other studies (101-104) deal with fully nonaqueous systems, especially those in which hydrogen bonding is a major type of interaction (30, 105, 106); these do not concern us here. Nakano studied the effect of aqueous methanol on the stability of the riboflavin-salicylate ion complex and found a decrease in stability with an increase in methanol concentration; he also studied the menadione-caffeine complex in 10% solutions of glycerin, methanol, acetonitrile, and acetone in water, and in all cases observed a decrease in stability relative to that observed in pure water (106). No medium effect studies had been instituted in these laboratories primarily because the results, whatever they might turn out to be, seemed of relatively low utility in accounting for the phenomena of interest. This is because no general model of molecular complex formation in solution was available (though many theories of molecular interactions have of

course been developed, and these can provide a guide for the interpretation of solvent effects). Recently a very simple thermodynamic model was suggested for the kinds of complex formation under consideration here (33), and as a consequence it was considered timely to initiate an investigation of some medium effects. This section describes these preliminary results. It is emphasized that these results are fragmentary and merely sketch out the nature of the effects.

1. Effect of Ionic Strength and Salts.

To evaluate the effects of ionic strength and specific ions on the stability of complexes the naphthalene-theophylline and methyl cinnamate-theophylline systems were studied. At pH 5.60 both ligand and substrate are electrically neutral, and are believed to form 1:1 complexes. The naphthalene-theophylline system has a large K_{11} value, which is helpful in providing sensitivity in detecting small changes.

The solubilities of naphthalene and of theophylline were studied in acetate buffers of varying ionic strengths. The data are plotted in Figure 24; as the concentration of ions in solution increases, the solubility decreases. The extrapolated value for naphthalene gives a solubility of 2.63×10^{-4} M, in water, and similarly the water solubility of theophylline

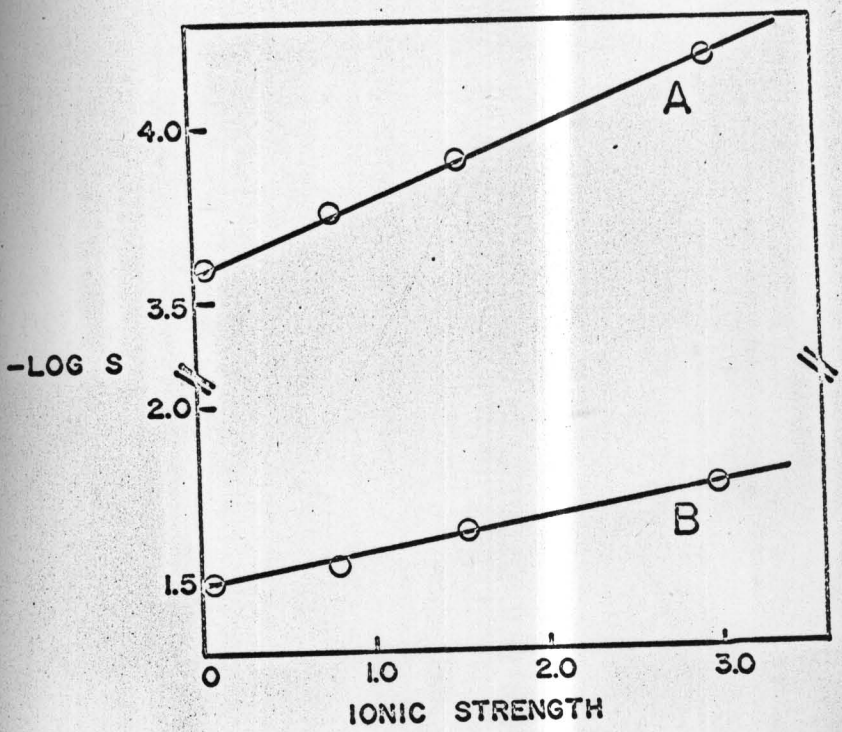


Figure 24. Plot of -log of solubility as a function of ionic strength. (A) Naphthalene; (B) Theophylline. 25.00; pH 5.60 sodium acetate buffer; ionic strength adjusted with NaCl.

is 3.23×10^{-2} M; these values compare favorably with literature values (2.40×10^{-4} M for naphthalene (107); 3.58×10^{-2} M in pH 6.75 phosphate buffer containing one per cent acetonitrile for theophylline (56b)). Beer's law plots of theophylline gave a molar absorptivity at 272 nm of 1.02×10^{4} for solutions of varying ionic strength (0.088 to 3.000 M), hence its molar absorptivity is essentially independent of ionic strength.

The interaction of naphthalene with theophylline was studied at several ionic strengths by the solubility method. The effect of specific ions was investigated at the same time; examples of the data obtained are given in Table XVI and Figure 25. The apparent stability constants are tabulated in Table XVII. The average of the stability constants for naphthalene-theophylline in sodium acetate buffer is $K_{11}' = 63.6\ \underline{M}^{-1}$ (standard deviation = 7.4). The reproducibility is poorer than the 10 - 15% that has been estimated from previous solubility studies (56b); the average, however, compares well with that obtained for phosphate buffer, $K_{11}' = 63.4\ \underline{M}^{-1}$. Changing from a monovalent to a divalent cation decreased the average K_{11}' to $52.6\ \underline{M}^{-1}$ (standard deviation = 0.7). In the methyl cinnamate-theophylline system, previous data gave a K_{11}' of $25\ \underline{M}^{-1}$ for phosphate buffer (56b), while a K_{11}' of $28.1\ \underline{M}^{-1}$ (standard deviation = 3.0) was obtained for the acetate buffer. This value is slightly

TABLE XVI

Apparent Solubility of Naphthalene at Various Theophylline Concentrations as a Function of Ionic Strength[a]

Theophylline concentration ($M \times 10^3$)	Naphthalene solubility ($M \times 10^4$) Ionic strength (M)		
	0.088	0.816	1.500
0	2.64	1.70	1.43
2	2.91	2.09	1.58
4	3.09	2.12	1.69
6	3.43	2.37	1.90
8	3.68	--	2.11
10	4.01	2.71	2.13
12	4.29	3.00	2.43
14	4.56	3.29	2.65
16	4.67	3.49	2.83
18	5.36	3.93	3.05
20	5.45	4.03	3.09

[a] 25.0°; pH 5.60 acetate buffer; ionic strength adjusted with NaCl.

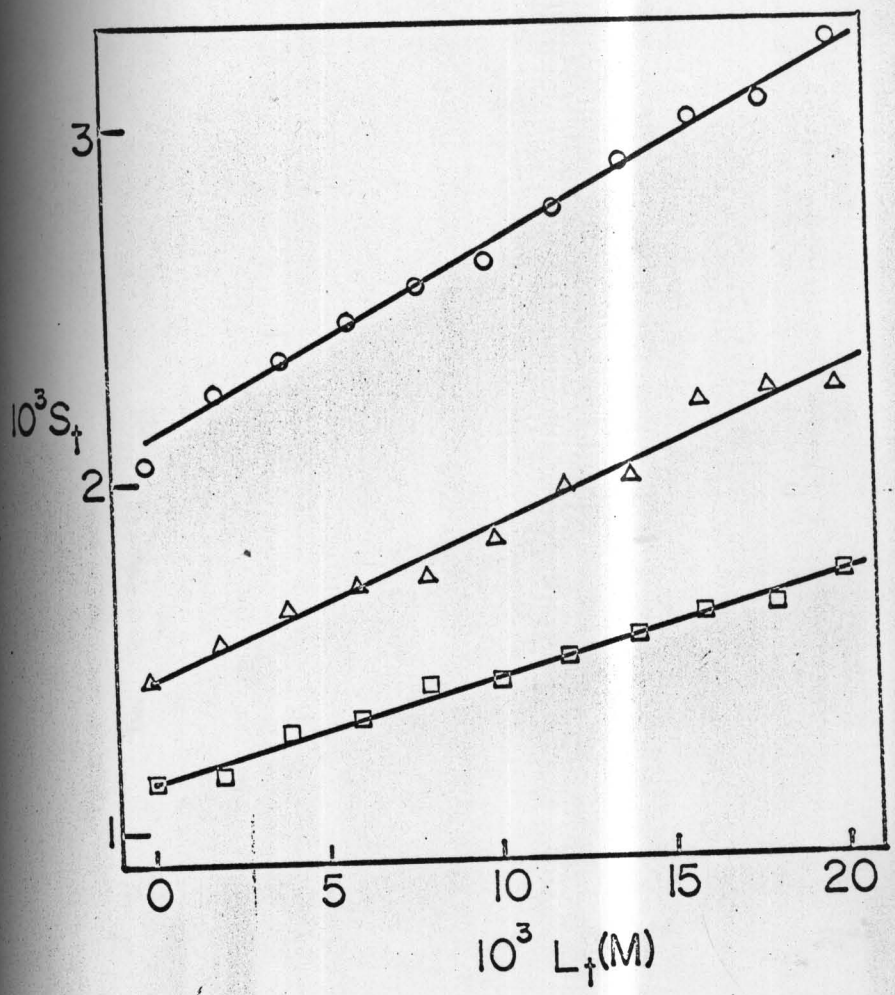


Figure 25. The apparent solubility of methyl cinnamate as a function of theophylline concentration at 25.0°; O ionic strength 0.088; △ ionic strength 0.816; □ ionic strength 1.500; pH 5.60 sodium acetate buffer; ionic strength adjusted with NaCl.

TABLE XVII

Apparent Stability Constants with Theophylline at 25.0°

Naphthalene

Cation	Anion	pH	μ (M)	K_{11}' (M^{-1})
Na	Acetate	5.60	0.088	55.6, 57.2
Na	Acetate	5.60	0.816	68.6, 72.9
Na	Acetate	5.60	1.500	63.6
Na	Phosphate	6.50	0.300	63.4*
Ca	Acetate	5.60	0.148	52.9
Ca	Acetate	5.60	0.262	51.9
Ca	Acetate	5.60	0.816	53.1

Methyl cinnamate

Cation	Anion	pH	μ (M)	K_{11}' (M^{-1})
Na	Acetate	5.60	0.088	26.4
Na	Acetate	5.60	0.816	31.7
Na	Acetate	5.60	1.500	26.3
Na	Phosphate	6.57	0.300	25.0 (56b)

*Personal communication, J. L. Cohen of this laboratory.

greater than that obtained with phosphate buffer; however, the difference is within the reproducibility of the method. Within the precision of the data, there appears to be no marked effect of ionic strength on the stability of these complexes.

2. Mixed Solvents.

The methyl cinnamate-theophylline system was studied by the spectral technique in methanol-water mixtures. The data obtained are shown in Figure 26; they show a decrease in complex stability constant with a decrease in the weight % water. As the concentration of methanol increases the K_{11} drops sharply, and the spectral change becomes too small for useful measurements.

Connors, et al. (33), on the basis of a simplified model, derived an equation to relate complex stability to area of molecular overlap.

$$\Delta G^{o} = A(G_{SL}^{o} - G_{MS}^{o} - G_{ML}^{o}) \qquad \text{(Eq. 14)}$$

where

ΔG^{o} = the standard free energy change for the process

$G_{SL}^{o}, G_{MS}^{o}, G_{ML}^{o}$ = the standard free energies of interaction per unit area between the "surfaces" designated by the subscripts

A = the area of overlap between substrate and ligand.

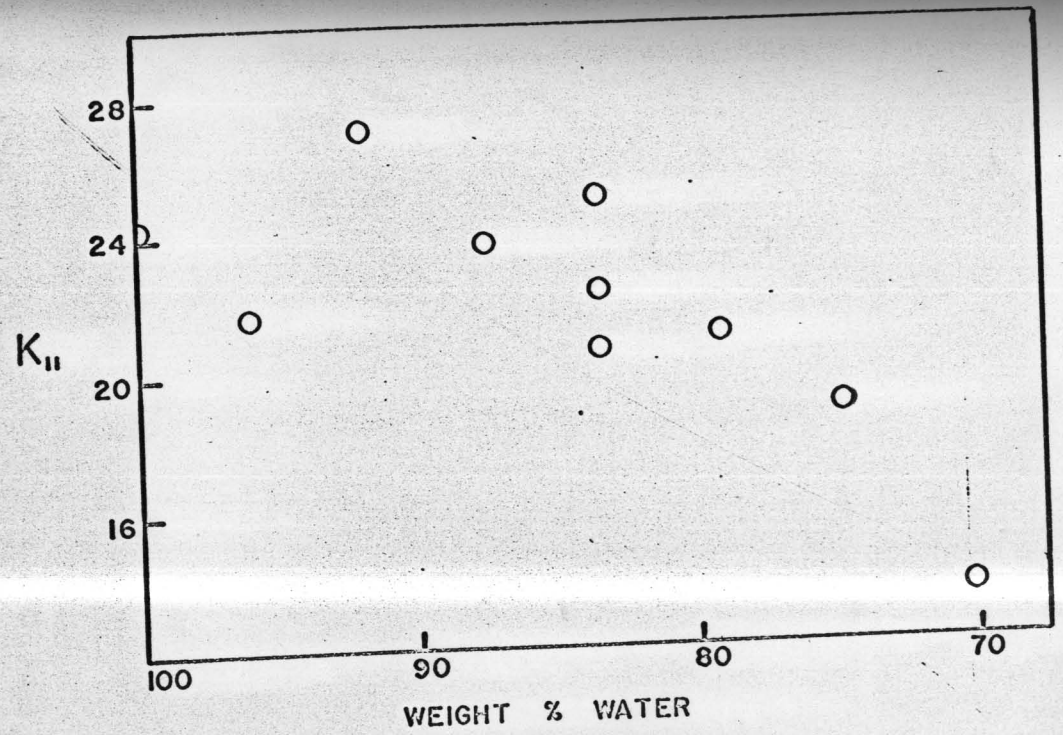


Figure 26. Spectral stability constants of methyl cinnamate-theophylline complex with change in weight % water at 25.0°.

They plotted $-\Delta G^{o}_{unitary}/N$ *vs* area of the smaller interactant for neutral complexes and found a slope value equivalent to 23.9 dynes/cm, which is the approximate magnitude of interfacial tensions between water and organic compounds. This observation suggests that the slope quantity may have some useful physical significance.

The data obtained in this study show that changing solvent has an effect on the stability of the complex. Assuming the area of overlap does not change upon change in solvent (and adopting the model), the difference must be due to an alteration in one or both of the free energy terms, G_{SM}^{o} and G_{LM}^{o}. For these two complexes, the decrease in the complex stability constant with an increase in methanol concentration suggests that the substrate-medium and ligand-medium interactions are more important than the substrate-ligand interaction in controlling complex stability. That is, if G_{SL}^{o} were the controlling factor, complex stability should not be markedly affected by changes in the medium.

It must be noted that these observations should not be extrapolated to complexes that are much different from the case studied. For example, charged complex species may respond to solvent changes quite differently. Interpretation of solvent effects probably will require a combination of a physical-chemical approach (in terms of solvent properties and forces of interaction) and an organic chemical viewpoint (in terms of specific atomic and group interactions).

DISCUSSION

A. Structural Implications of the Reactivity of Xanthine-Acetoxycinnamic Acid Anion Complexes.

Earlier work (1, 36, 44) with cinnamate-xanthine complexes had defined in part the areas of the molecule that are required for this complex formation. In these studies it was found that complexation, by such ligands as theophyllinate, can essentially completely inhibit nucleophilic attack at the cinnamate ester group by hydroxide ion. This kinetic method of studying complexation equilibria involves a non-thermodynamic study of a system in which the complexation process is at equilibrium. The method can be utilized to provide information on the juxtaposition of groupings in the complex and to give insight into the electronic changes caused by interaction.

We studied the effect of theophyllinate on the ortho-, meta- and para-acetoxycinnamic acid anions (substituted phenyl acetates). In this substrate the labile ester group is at the opposite end of the molecule from all substrates in prior experiments that utilized cinnamate esters. Within this series we anticipated no variation in complexing tendency, but we did expect that the complexed esters would retain some of their reactivity towards hydroxide ion attack. The goal was to establish

the overlap limits of the ligand at the phenyl ring portion of cinnamate substrates.

We found K_{11} values (7.5, 10.5, 14 M^{-1}) similar to those anticipated. However, the reactivity of these esters was not completely lost upon complexation (as predicted), which is in marked contrast to the case where the carboxylic end of the molecule is esterified, and we obtained q_{11} values of 0.4. This suggests that the theophyllinate molecule is bound to the -CH=CH-COOR portion of the cinnamate molecule, but does not envelope the phenyl ring. The implications of the results in this paragraph will be developed in the following sections.

1. The nature of q_{11}.

The $q_{11} \approx 1$ values for the cinnamate esters were interpreted to mean that the ligand was directly involved in interaction at the ester site (44). Both of the molecules are planar and evidence has been presented to show that they interact in a plane-to-plane manner (33). If the ligand lies immediately opposite the functional group undergoing reaction on the substrate, evidently steric factors can largely control q_{11}. Thus an attacking hydroxide ion approaches an ester group perpendicular to the plane of the carboxyl group (108). If one side of the group is "protected" by a complexed ligand, q_{11} must be not smaller than 0.5 by a purely steric effect, which

can be interpreted on a statistical basis. In fact, observed q_{11} values are always larger than 0.5 for these systems, suggesting additional rate retarding effects (44).

The q_{11} values of 0.4 for the acetoxycinnamic acids are therefore rationalized as polar effects, the assumption being made that almost all cinnamates form roughly the same type of complex with the same ligand. Thus, in this type of complex, we consider that the ligand lies far enough from the reaction site so that steric effects are substantially absent.

We can represent the acetoxycinnamic acid anion (S)-theophyllinate (L) system as

$$S + L \underset{}{\overset{K_{11}}{\rightleftharpoons}} SL \qquad \text{(Eq. 15)}$$

$$S + \ominus OH \xrightarrow{k_s} \text{products} \qquad \text{(Eq. 16)}$$

$$SL + \ominus OH \xrightarrow{k_{11}} \text{products} \qquad \text{(Eq. 17)}$$

where K_{11} is the complex stability constant and k_s and k_{11} are the second-order rate constants for the reaction of free substrate and complexed substrate, respectively. From the experiments in which the apparent rate constant k_s' was measured as a function of ligand concentration we obtained the quoted K_{11} and q_{11} values, where $q_{11} = 1 - k_{11}/k_s$. We may ask: What does a q_{11} value of 0.4 correspond to in terms of increase in activation

energy? From transition-state theory we write for the two rate constants:

$$k_{11} = (\frac{k_T}{h})e^{-\Delta G_{11}^{\neq}/RT} \qquad \text{(Eq. 18)}$$

$$k_s = (\frac{k_T}{h})e^{-\Delta G_s^{\neq}/RT} \qquad \text{(Eq. 19)}$$

where h is Planck's constant, k is Boltzmann's constant, $\Delta G_{11}^{\neq}$ is the free energy of activation for reaction of the complexed substrate, $\Delta G_s^{\neq}$ is the free energy of activation of the substrate, R is the gas constant, and T is the absolute temperature. Upon substitution of equations (18) and (19) into the definition of q_{11} we obtain

$$\delta_R \Delta G^{\neq} = -2.303RT \log (1 - q_{11}) \qquad \text{(Eq. 20)}$$

where $\delta_R \Delta G^{\neq} = \Delta G_{11}^{\neq} - \Delta G_s^{\neq}$. Calculations of $\delta_R \Delta G^{\neq}$ for T = 298.16° K are given in Table XVIII and the nature of this function is shown graphically in Figure 27.

From Figure 27 it is important to note the very small differences in free energy of activation between SL and S that can lead to appreciable q_{11} values; in fact, q_{11} values of up to 0.6 can be produced by changes in

TABLE XVIII

Relationship of $\delta_R \Delta G^{\neq}$ to q_{11} at 25.0°C

q_{11}	$\delta_R \Delta G^{\neq}$ (kcal/mole)[a]
0.0	0
0.1	0.0625
0.2	0.132
0.3	0.212
0.4	0.303
0.5	0.411
0.6	0.543
0.7	0.715
0.8	0.755
0.9	1.365
0.95	1.777
0.97	2.080
0.99	2.729
0.999	4.094

[a]Calculated with equation (20).

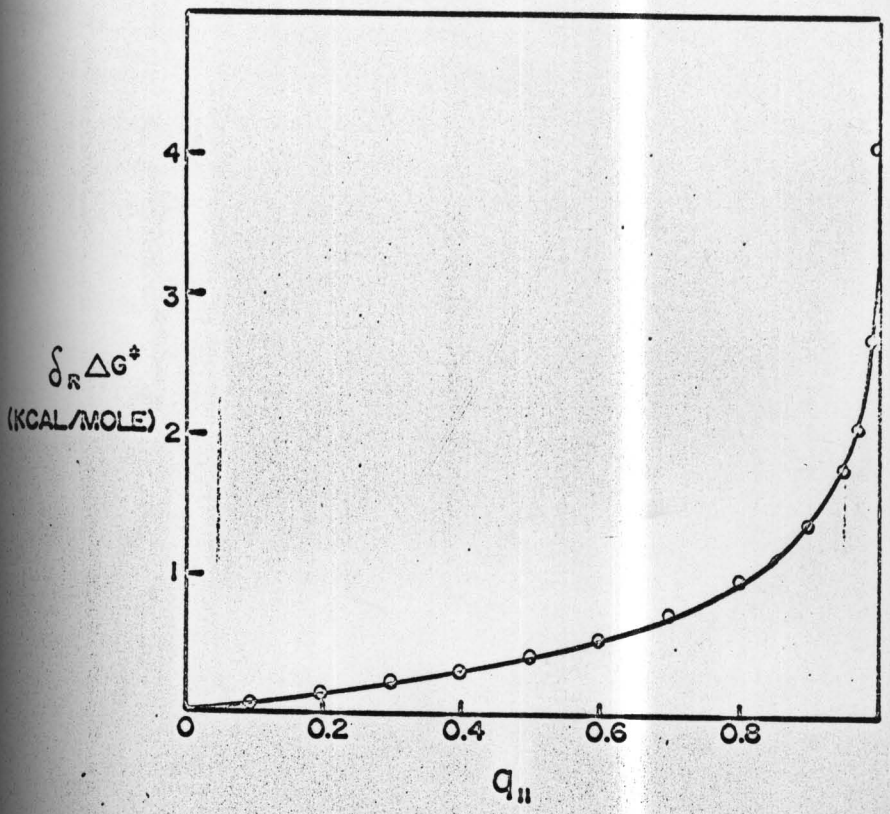


Figure 27. Relationship of $\delta_R \Delta G^{\ddagger}$ to q_{11}, where $q_{11} = 1 - k_{11}/k_s$ and $\delta_R \Delta G^{\ddagger} = \Delta G_{11}^{\ddagger} - \Delta G_s^{\ddagger}$. $T = 25.0^{\circ}$.

$\delta_R \Delta G^{\neq}$ smaller than RT. Thus we can understand that q_{11} values of 0.4 could be caused by an extremely minor energy perturbation. If such minor energy effects may have major consequences on the q_{11} values, the accuracy of experimental estimates of q_{11} may be related to the value of q_{11} and it probably is best to quote q_{11} for the acetoxycinnamic acid anion-theophyllinate system as 0.3-0.5.

These q_{11} values of less than 0.6 for intermolecular nucleophilic attack on a complexed molecule are the first to be recorded. Kramer and Connors (44) have observed q_{11} values of 0.6-0.9 for the p-nitrophenyl benzoate-theophylline system with various attacking nucleophiles. (hydroxylamine, hydrazine, hydrogen peroxide anion, and sulfite dianion). They speculate that complexation of a substrate may stabilize the initial state of the system and may also raise the transition state energy. In addition, intermolecular interactions between the substrate and ligand may produce electronic perturbations that could extend to the unblocked site of the ester group and this effect could result in further initial-state alterations (for a more complete discussion see reference 44).

Note also, in Figure 27, that the energy difference rises abruptly as q_{11} approaches unity. In earlier work q_{11} values of "essentially unity" have been recorded.

The experimental uncertainty in q_{11} of 0.1 unit (44) corresponds, at q_{11} values above 0.9, to very large energies. Figure 27 makes very apparent the need for, and probable utility of, more accurate q_{11} determinations in the high q_{11} range.

2. Structure-ligand relationship in the complexes.

For the ortho-, meta-, and para-acetoxycinnamic acid anions with theophyllinate, we have obtained q_{11} values of 0.4, indicating that the acetoxy group must not be sterically hindered and does not participate in direct interaction with the ligand, as either of the possibilities would result in high q_{11} values. A high q_{11} value, approximately equal to one, was observed for the p-chlorophenyl acetate without a cinnamate structure where the ligand interacts directly with the labile ester group.

The common overlap areas of substrate and ligand should be considered first. Several points bearing on this have previously been demonstrated (33, 44, 56b), and the present work adds another:

(1) A correlation between the free energy change for complex formation and the planar area of the smaller of the two interactants has been demonstrated (33). This implies that these 1:1 complexes have a plane-to-plane orientation.

(2) Kinetic studies on the complexed benzoate and cinnamate substrates subjected to attack by hydroxide ion give q_{11} values close to unity (44, 56b). This is interpreted to mean that in the complex the ligand must be very near the ester group.

(3) Attack of the double bond of a cinnamate complex by sulfite is essentially completely inhibited (44); therefore the ligand must lie near this portion of the substrate molecule.

(4) Variations in X in a series of cinnamic acid derivatives, $C_6H_5CH{=}CHCOX$, result in only minor changes in K_{11} with theophylline; it is inferred that X is not strongly involved in the intermolecular interaction (33).

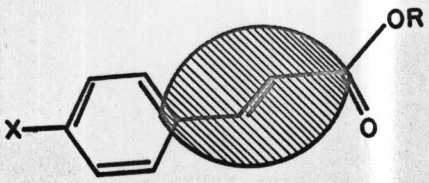
(5) Replacement of the phenyl group by a methyl group in $C_6H_5CH{=}CHCOOCH_3$ leads to a marked decrease in complexing tendency; this implicates the ring in the complex interaction.

Finally, from the present work:

(6) Theophylline complexes with ortho-, meta-, and para-acetoxycinnamic acid anions to give K_{11}' values of 7.5, 10.5 and 14 M^{-1}, respectively, and q_{11} values of about 0.4. (Similar results are obtained with related substrates, such as p-acetoxybenzalacetone.)

It seems likely that some involvement of the o-acetoxy group with the ligand in the complex formation may lead to the somewhat higher K_{11}' value. The values of 7.5 M^{-1} and 10.5 M^{-1} for the para and meta substrates are not significantly different, and compare well with the 7.2 M^{-1} found for unsubstituted cinnamate ion (109). On the other hand, the low q_{11} values suggest essentially no steric hindrance to attack at any position. Molecular models indicate that these conclusions are reasonable. The acetoxy substituent cannot be coplanar with the phenyl ring, and in the ortho compound this group is practically perpendicular to the plane of the ring. Interaction between the oxygen bonded to the ortho ring position and the ligand is possible while leaving the carbonyl group (which is on the other side of the molecule) available to be attacked.

The total picture of a cinnamate-xanthine complex, drawn on the basis of the above points, looks like structure 4, where the ligand molecule is represented by the shaded area.



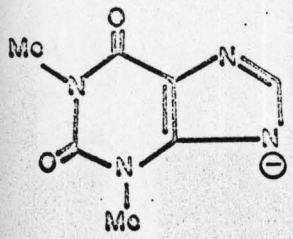
4

This is the first-order result for the present approach to the elucidation of complex structures in solution. It is important to note that substantial modifications in the basic cinnamate structure (ionization, esterification, ring substitution) have introduced no inconsistent patterns in the complexing evidence. That is, the present work tends to substantiate the working assumption that it is reasonable to think of a complex structure when comparing similar substrates and ligands. (Minor differences must of course be present, but these are not serious enough to introduce inconsistencies in the first-order picture).

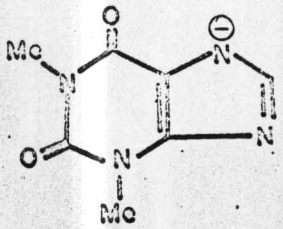
The next level of understanding in the present study involves the relative orientations of the "ends" of the molecules. One may ask: Is the 5-membered ring or the 6-membered ring of the xanthine lying closer to the phenyl ring of the cinnamate? This question cannot yet be answered, but some of the pertinent points can be described. Consider the electronic distribution in theophyllinate.

Structures 5, 6 and 7 should make the greatest contribution because oxygen and nitrogen are more electronegative than carbon. Thus, it appears that the negative charge is distributed over the 5-membered ring plus the adjacent oxygen.

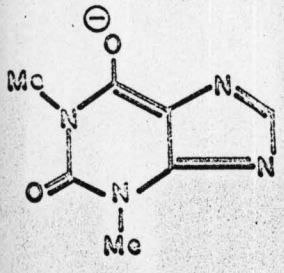
It seems therefore that in the complex, the 5-membered ring may lie furthest from the ionized $COO^{\ominus}$



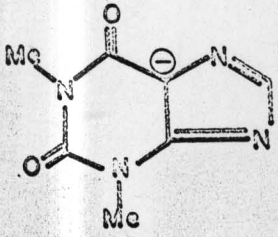
5



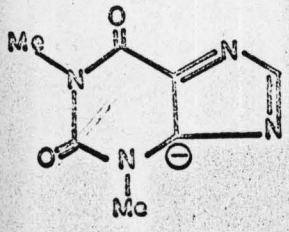
6



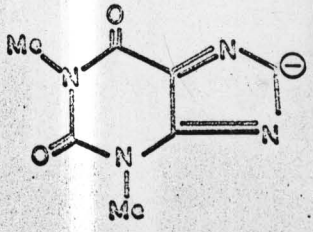
7



8



9



10

group of the ester. This places the 5-membered ring in the vicinity of the phenyl ring with the 6-membered ring of the theophyllinate near the $COO^{\ominus}$ group. In this configuration both the para and the meta, and perhaps the ortho, positions are essentially free of steric hindrance by the ligand (consistent with the low q_{11} values).

Possible complex structures utilizing these requirements are shown in Figure 28. While the proposed model is consistent with all of the data obtained, it must be kept in mind that minor energy perturbations or relative minor changes of an interactant might lead to substantially different complex structures.

B. Catalytic Effects of Some Xanthine-Methyl Acetoxycinnamate Systems.

1. Selection of ligands.

Initially a significant hydrolysis of p-acetoxybenzalacetone by 7-(2,3-dihydroxypropyl)-theophylline was observed; we felt that it was possible that 7-(2,3-dihydroxypropyl)-theophylline could catalyze the hydrolysis of methyl p-acetoxycinnamate by the same mechanism as with p-acetoxybenzalacetone. Examination of C.P.K. models showed that the 3-hydroxy group could be in a position to function catalytically or by slightly altering the complex structure, the 2-hydroxy

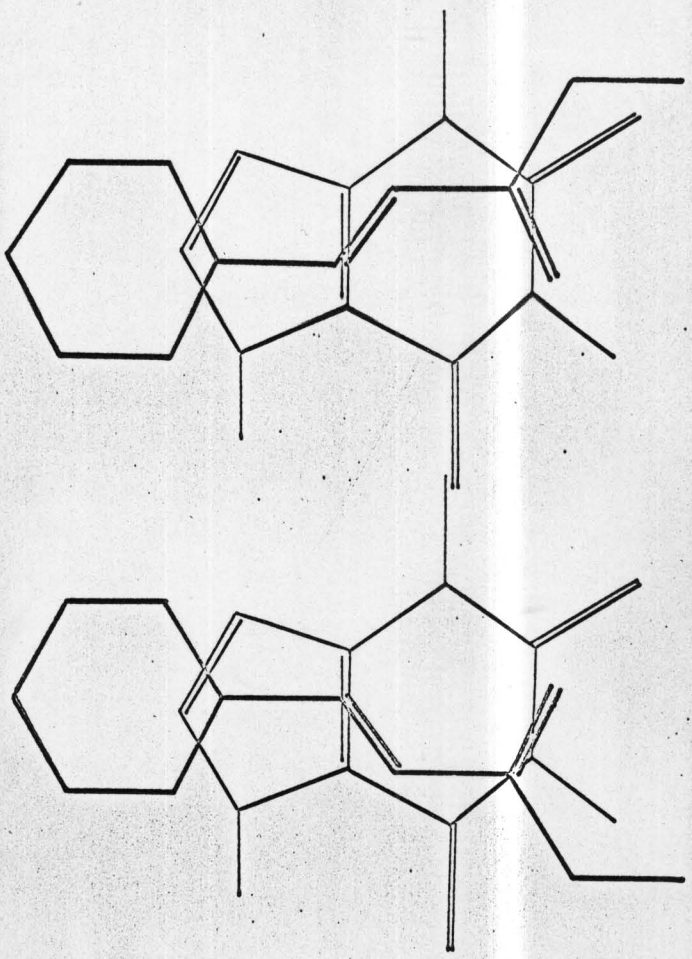


Figure 28. Some proposed structures for the methyl cinnamate-theophyllinate complex in aqueous solution.

group could be brought into a position for attack. Further examination of the models suggested that if the catalysis involved complex formation, with intra-complex attack by one of the hydroxyl groups, then it was probable that 7-(2,3-dihydroxypropyl)-theophylline would not catalyze the hydrolysis of methyl o-acetoxycinnamate or methyl m-acetoxycinnamate. This supposition, however, was not borne out by experimental studies.

In order to determine what component of the 7-(2,3-dihydroxypropyl)-theophylline structure was required for catalysis, several other 7-substituted theophyllines were prepared. The K_{11}' values obtained for methyl p-acetoxycinnamate were 10 M^{-1} with 7-(n-propyl)-theophylline, 5 M^{-1} with the 7-(2-hydroxypropyl)-theophylline, and 5 M^{-1} with the 7-(3-hydroxypropyl)-theophylline. In these cases the 7-substituted theophyllines inhibited alkaline hydrolysis and gave q_{11} values of less than 0.7. No significant difference was seen for the inhibition by the 7-(2-hydroxypropyl)-theophylline or the 7-(3-hydroxypropyl)-theophylline; a greater inhibition was observed for the 7-(n-propyl)-theophylline compound. It is not possible to make any definite statements relating to the K_{11} values for these ligands separately as it is quite probable that multiple complexes can be formed. This has already been shown for the 7-(n-propyl)-theophylline ligand (62). Kramer has shown that 7-(2,3-dihydroxypropyl)-theophylline with

trans-cinnamoylsalicylic acid does not form multiple complexes (44). Hence the inhibition data are consistent, but do not substantiate the original speculation that one or the other hydroxyl group may be responsible for the catalysis. Approximately eight per cent catalysis of hydrolysis of methyl p-acetoxycinnamate was observed in 0.2 M propylene glycol; however, this could be attributed to a solvent effect. Hence the catalysis appears to be unique to 7-(2,3-dihydroxypropyl)-theophylline. The above results are summarized in Figure 29.

Comparison of the kinetically obtained K_{11}' of 2 M^{-1} with that obtained for a spectral study of methyl p-acetoxycinnamate with 7-(2,3-dihydroxypropyl)-theophylline, $K_{11}' = 11\ M^{-1}$, indicates that the simple scheme that we proposed for analysis of the system cannot be correct as an equivalent complex stability constant should be obtained by the spectral and kinetic techniques, since if an analogy between the cinnamoylsalicylic acid system and this one can be drawn, no multiple complexing occurs. A more complicated scheme will be considered in the following section.

2. Some possible interpretations.

Since all previous complexes of methyl cinnamates with xanthines have shown inhibition of

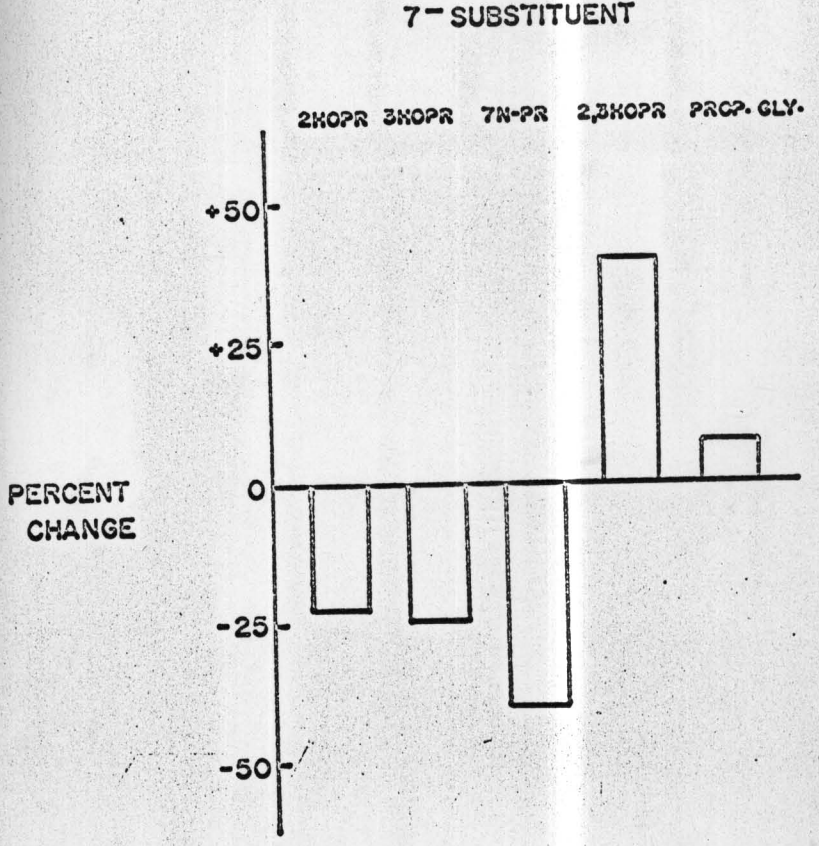


Figure 29. Percent change in hydrolysis of methyl p-acetoxycinnamate with 0.2 M 7-substituted theophyllines and 0.2 M propylene glycol; pH 10.8; ionic strength 0.3; 25.0°.

reactions, it is probable that the catalysis observed for 7-(2,3-dihydroxypropyl)-theophylline is a composite of concurrent catalysis and inhibition.

A possible scheme may be outlined as follows:

(1) complexation;

(2) alkaline hydrolysis of uncomplexed substrate;

(3) alkaline hydrolysis of complexed substrate;

(4) intramolecular catalysis by the ligand in the complex.

The rate expressions may be written

$$-\frac{dS}{dt} = k_s(S)(OH^-) + k_{11}(SL)(OH^-) + k_c(SL)(OH^-) \qquad \text{(Eq. 21)}$$

$$-\frac{dS}{dt} = k_{obs}\, S_t \qquad \text{(Eq. 22)}$$

We define f_{11} as the fraction of the substrate present as complex or

$$f_{11} = \frac{K_{11}(L)}{1 + K_{11}(L)} \qquad f_{11} + f_s = 1 \qquad \text{(Eq. 23)}$$

and

$$k_s' = \frac{k_{obs}}{OH^-} \qquad \text{(Eq. 24)}$$

Combining with the above rate expressions

$$(k_s' - k_s) = f_{11}(k_{11} + k_c - k_s) \qquad \text{(Eq. 25)}$$

We can define

$$q_{11} = 1 - \frac{k_{11}}{k_s} \quad \text{so} \quad \frac{k_{11}}{k_s} = 1 - q_{11} \qquad \text{(Eq. 26)}$$

and

$$p_{11} = \frac{k_c}{k_s} - 1 = \frac{k_c - k_s}{k_s} \qquad \text{(Eq. 27)}$$

Therefore,

$$\frac{k_s' - k_s}{k_s} = f_{11}(1 - q_{11} + p_{11}) \qquad \text{(Eq. 28)}$$

Replacing f_{11} and inverting,

$$\left(\frac{k_s}{k_s' - k_s}\right)_{obs} = \frac{1}{(1 - q_{11} + p_{11})K_{11}(L)} + \frac{1}{(1 - q_{11} + p_{11})} \qquad \text{(Eq. 29)}$$

According to equation (29) the original data should give a plot with the Y-intercept equal to $1/(1 - q_{11} + p_{11})$ and the X-intercept $-K_{11}$. The data are presented in Figure 16 and unfortunately do not bear this out, since K_{11} from this plot (2 M^{-1}) is markedly different from the 11 M^{-1} found spectrally.

An alternate scheme may be outlined:

(1) complexation;

(2) alkaline hydrolysis of uncomplexed substrate;

(3) alkaline hydrolysis of complexed substrate;

(4) intermolecular catalysis by the 7-(2,3-dihydroxypropyl)-theophylline anion of the uncomplexed substrate;

(5) intermolecular catalysis by the 7-(2,3-dihydroxypropyl)-theophylline of the complex.

We can write the rate expression:

$$-\frac{dS}{dt} = k_s(S)(OH^-) + k_{11}(SL)(OH^-) + k_s^L(S)(L)(OH^-) + k_{11}^L(SL)(L)(OH^-) \qquad \text{(Eq. 30)}$$

$$-\frac{dS}{dt} = k_{obs}\, S_t \qquad \text{(Eq. 31)}$$

and define the q values

$$q_{11} = 1 - \frac{k_{11}}{k_s} = \frac{k_s - k_{11}}{k_s} \qquad \text{(Eq. 32)}$$

$$q_{11}^L = 1 - \frac{k_{11}^L}{k_s^L} = \frac{k_s^L - k_{11}^L}{k_s^L} \qquad \text{(Eq. 33)}$$

Continuing the definition for f_{11} and k_s', we obtain

$$k_s' = k_s f_s + k_{11} f_{11} + k_s^L f_s(L) + k_{11}^L f_{11}(L) \qquad \text{(Eq. 34)}$$

Substitution and rearrangement yields

$$\left(\frac{k_s}{k_s' - k_s}\right)_{obs} = \frac{k_s}{\{k_s^L - q_{11}k_sK_{11} + k_s^LK_{11}(L)[1 - q_{11}^L]\}(L)}$$

$$+ \frac{k_sK_{11}}{k_s^L - q_{11}k_sK_{11} + k_s^LK_{11}(L)[1 - q_{11}^L]} \qquad \text{(Eq. 35)}$$

In the above equation the slope and intercept are both functions of (L), and therefore equation (35) is not the equation of a straight line.

However, in the special case that $q_{11}^L = 1$, then equation (35) becomes

$$\left(\frac{k_s}{k_s' - k_s}\right)_{obs} = \frac{k_s}{(k_s^L - q_{11}k_sK_{11})(L)} + \left(\frac{k_sK_{11}}{k_s^L - q_{11}k_sK_{11}}\right) \qquad \text{(Eq. 36)}$$

Under these conditions we have a straight line. But since q_{11} is probably 0.3-0.5, it is unlikely that $q_{11}^L = 1$. Thus if rate equation (30) defines the system, it follows that equation (35) is applicable. Therefore the anomalous K_{11} of 2 that was evaluated kinetically is conceivable; in fact we cannot make this plot. Perhaps we can even regard the disagreement between 2 (kinetic) and 11 (spectral) as evidence that this

scheme (or another one similar to it) is operative. Additional evidence for inter molecular direct attack is the lack of ortho, meta, para specificity in the 7-(2,3-dihydroxypropyl)-theophylline catalyses of the methyl acetoxycinnamates, see Table X.

If the catalytic effect were due to free 7-(2,3-dihydroxypropyl)-theophylline, then this catalysis would be effective at any pH. Thus by lowering the pH we would reduce the extent of hydroxide ion catalyzed hydrolysis without reducing the 7-(2,3-dihydroxypropyl)-theophylline catalysis or a complex mediated reaction. In a separate study the rate of hydrolysis at pH 8.5 was investigated and only inhibition was observed at this pH. This is consistent with a catalytic rate term involving 7-(2,3-dihydroxypropyl)-theophylline anion or a kinetically equivalent term (as in equation (30)).

The literature did not contain the pK_a values for the hydroxypropyl theophyllines. The approximate pK's for model alcohols (110, 111) are 16.8 for 2-propanol, 16.3 for 1-propanol and 14.7 for propylene glycol (1,2-dihydroxypropane). There are two orders of magnitude difference between these numbers, and if our assumption that the theophylline portion in the 7-(2-hydroxypropyl)-theophylline, 7-(3-hydroxypropyl)-theophylline, and the 7-(2,3-dihydroxypropyl)-theophylline exerts the same effect, it may be possible to explain the

similarity of inhibition by the 7-(2-hydroxypropyl)-theophylline and 7-(3-hydroxypropyl)-theophylline and the catalysis observed by 7-(2,3-dihydroxypropyl)-theophylline. That is, at a given pH a substantially larger fraction of the 7-(2,3-dihydroxypropyl)-theophylline is in the oxyanion form relative to either of the monohydric compounds. The anion is the attacking nucleophile. This may also be substantiated by the observation that the anion of theophylline-1-acetic acid exhibited inhibition of the acetoxycinnamic acids because of its lower nucleophilicity.

From all previous studies, we observe that inhibition effects are more general than catalytic effects via complexing, but the latter are potentially more powerful in locating juxtaposed features on the substrate and ligand.

C. Substituent Effects in Theophylline-Methyl Cinnamate Complexes.

Work on the theophylline complexes of meta- and para-substituted methyl cinnamates reveals that K_{11} is not significantly different for these ten substrates.

The attempted correlation of Hammett's substituent constants with the complex stability constant failed, as did an attempted correlation with dipole moments. Small structural modifications do not have an appreciable effect

on K_{11} for this system. Such effects as are observed are small second-order effects and their interpretation is not presently possible.*

If our assumptions about complex structure from part A above are correct, these results provide further examples in which the ligand apparently does not extensively overlap the phenyl ring of the cinnamate complex.

One interpretation of the insensitivity of complex stability to ring substitution is based upon the correlation described earlier between complex stability and overlap area. Based on a simple model this equation was derived:

$$\Delta G^{\circ} = A(G_{SL}^{\circ} - G_{MS}^{\circ} - G_{ML}^{\circ})$$

As the solvent effect study implied, the medium plays an important role in determining complex stability. If the two medium interaction terms, G_{MS}° and G_{ML}°, are much more important than the substrate-ligand term G_{SL}°, then even substantial changes in substrate structure might have little overall effect on ΔG°, as in fact we observe, in the present series of substrates.

*When a substituent on a molecule fails to change the equilibrium constant for a reaction, at least two conclusions can be drawn: (1) the equilibrium has been studied at the isoequilibrium temperature (112); and (2) the equilibrium does not involve an interaction between the equilibrium site and substituent site.

D. Summary.

Utilizing kinetic inhibitions, earlier work with cinnamate-xanthine complexes (33, 44, 56b) had defined in part the areas of the molecule that are required for complex formation. It remained however, to examine the role in complexation of substituents on the phenyl ring of cinnamates. The principal investigation was of the reactivities of ring-substituted acetoxy derivatives of cinnamic acid. This study has been extended by investigation of the effects of electron-donating and electron-withdrawing substituents on the complexing tendency of carboxylic acid derivatives with theophylline and its anion. The study was further extended by investigating the effect of variation of the 7-substituent of substituted theophyllines on complexation and reactivity with a single substrate.

The ortho-, meta-, and para-acetoxycinnamic acid anions were studied in the presence of theophylline anion. Unlike previous cinnamate-theophylline anion complexes, the complexed esters retained some of their reactivity towards hydroxide ion attack. This, along with previous knowledge of these systems, suggested that the theophylline anion was intimately bound to the -CH=CH-COOR portion of the cinnamate molecule and did not envelop the phenyl ring.

Studies with a series of *para* and *meta* substituted ($-NO_2$, $-OH$, $-OCH_3$, $-CH_3$, $-Cl$, $-Br$, $-OOCCH_3$) methyl cinnamates with theophylline were carried out by spectral analysis. These small structural modifications did not lead to marked changes in the complex stability constant for these systems. This work further substantiates the above conclusion that the ligand does not extensively overlap the phenyl ring of the cinnamate in the complex.

With methyl *p*-acetoxycinnamate as substrate, the R_7 group on theophylline was varied: R_7 = *n*-propyl, 2-hydroxypropyl, 3-hydroxypropyl, and 2,3-dihydroxypropyl. For all ligands except 7-(2,3-dihydroxypropyl)-theophylline inhibition of the acetoxy group reactivity was observed upon complexation. These complexes also retained some of their reactivity towards hydroxide attack. The catalysis observed with the 2,3-dihydroxypropyl compound was attributed to direct intermolecular attack, and some possible interpretations have been given.

On the strength of the above findings some possible structures for the complex between cinnamates and theophylline were proposed.

BIBLIOGRAPHY

1. Connors, K. A. and Mollica, J. A., Jr., J. Pharm. Sci., 55, 772 (1966).

2. Rose, J., "Molecular Complexes," Pergamon Press, N.Y., 1967.

3. Andrews, L. J. and Keefer, R. M., "Molecular Complexes in Organic Chemistry," Holden-Day, Inc., San Francisco, 1964.

4. Briegleb, G., "Elektronen-Donator-Acceptor Komplexe," Springer-Verlag, Berlin, 1961.

5. Higuchi, T. and Zuck, D. A., J. Am. Pharm. Assoc., Sci. Ed., 41, 10 (1952).

6. Higuchi, T. and Zuck, D. A., ibid., 42, 138 (1953).

7. Higuchi, T. and Lachman, L., ibid., 44, 521 (1955).

8. Lachman, L. and Higuchi, T., ibid., 46, 32 (1957).

9. Lachman, L., Guttman, D. E. and Higuchi, T., ibid., 46, 36 (1957).

10. Wadke, D. A. and Guttman, D. E., J. Pharm. Sci., 54, 1293 (1965).

11. Guttman, D. E., J. Pharm. Sci., 51, 1162 (1962).

12. Chin, T. F., Chung, P. H. and Lach, J. L., J. Pharm. Sci., 57, 44 (1968).

13. Van Etten, R. L., Clowes, G. A., Sebastian, J. F. and Bender, M. L., J. Am. Chem. Soc., 89, 3253 (1967).

14. Levy, G. and Reuning, R. H., J. Pharm. Sci., 53, 1471 (1964).

15. Reuning, R. H. and Levy, G., ibid., 56, 843 (1967).

16. Reuning, R. H. and Levy, G., ibid., 57, 1335 (1968).

17. Reuning, R. H. and Levy, G., ibid., 57, 1342 (1968).

18. Reuning, R. H. and Levy, G., ibid., 58, 79 (1969).

19. Meyer, M. C. and Guttmam, D. E., ibid., 57, 245 (1968).

20. Woolley, D. W., Hershey, J. W. B. and Koehelik, I. H., Proc. Natl. Acad. Sci. U.S.A., 48, 709 (1962).

21. Anderson, B. M., Cordes, E. H. and Jencks, W. P., J. Biol. Chem., 236, 455 (1961).

22. Bruice, T. C., Fife, T. H., Brown, J. J. and Brandon, N. E., Biochemistry, 1, 7 (1962).

23. Bennett, G. M. and Willis, G. H., J. Chem. Soc., 256 (1929).

24. Martin, A. N., "Physical Pharmacy," Lea and Febiger, Philadelphia, 1963.

25. Higuchi, T. and Lach, J. L., J. Am. Pharm. Assoc., Sci. Ed., 43, 349 (1954).

26. Higuchi, T. and Lach, J. L., ibid., 43, 524, 527 (1954).

27. Dewar, M. J. S. and Thompson, C. C., Jr., Tetrahedron, Supp. No. 7, 97 (1966).

28. Mulliken, R. S., J. Am. Chem. Soc., 74, 811 (1952).

29. Mulliken, R. S., Rec. trav. Chim., 75, 845 (1956).

30. Nakano, M., Nakano, N. I. and Higuchi, T., J. Phys. Chem., 71, 3954 (1967).

31. Murrell, J., J. Am. Chem. Soc., 81, 5037 (1959).

32. Nakano, M. and Higuchi, T., J. Pharm. Sci., 57, 183 (1968).

33. Connors, K. A., Infeld, M. H. and Kline, B. J., J. Am. Chem. Soc., 91, (in press) (1969).

34. McClellan, A. L., "Tables of Experimental Dipole Moments," W. H. Freeman, San Francisco, 1963.

35. Sutton, L. E., in "Determination of Organic Structures by Physical Methods," E. A. Braude and F. C. Nachod, eds., Academic Press, N.Y., 1955.

36. Kline, B. J., Ph.D. dissertation, University of Wisconsin, Madison, Wisconsin, 1968.

37. Donbrow, M. and Jan, Z. A., J. Pharm. Pharmacol., 17, Supp., 129S (1965).

38. Andrews, L. J., Chem. Rev., 54, 713 (1954).

39. Eide, G. J. and Speiser, P., Acta Pharm. Suecica, 4, 185, 201 (1967).

40. Brooke, D. and Guttman, D. E., J. Pharm. Sci., 57, 1206 (1968).

41. Davidson, S. J. and Jencks, W. P., J. Am. Chem. Soc., 91, 225 (1969).

42. Ross, S. D. and Labes, M. M., ibid., 77, 4916 (1955).

43. Zincke, Th. and Leisse, Fr., Ann., 322, 224 (1902).

44. Kramer, P. A. and Connors, K. A., J. Am. Chem. Soc., 91, 2600 (1969).

45. Ts'o, P. O. P. and Chan, S. I., ibid., 86, 4176 (1964).

46. Powell, H. M., Huse, G. and Cooke, P. W., J. Chem. Soc., 153 (1943).

47. Kosower, E. M., "Reactions Through Charge-Transfer Complexes," in S. A. Cohen, A. Streitwieser, Jr. and R. W. Taft, eds., "Progress in Physical Organic Chemistry," Vol. 3, Interscience, N.Y., 1965.

48. Pullman, B. and Pullman, A., Proc. Natl. Acad. Sci., U.S.A., 44, 1197 (1958).

49. Schnaare, R. S. and Martin, A. N., J. Pharm. Sci., 54, 1707 (1965).

50. Eckert, Th., Arch. Pharm., 295, 232 (1962).

51. Damiani, A., DeSantis, P., Giglio, E., Liguori, A. M., Puliti, R. and Ripamonti, A., Acta Cryst., 19, 1048 (1967).

52. Aso, C., Kunitake, T. and Shinkai, S., Chem. Commun., 1483 (1968).

53. Shefter, E., J. Pharm. Sci., 57, 1163 (1968).

54. Shefter, E., ibid., 57, 350 (1968).

55. Arnone, A. and Marchessault, R. H., "Molecular Association in Biological and Related Systems," in R. F. Gould, ed., "Advances in Chemistry" series no. 84, American Chemical Society, Washington, D.C., 1968.

56a. Mollica, J. A., Jr. and Connors, K. A., J. Pharm. Sci., 55, 772 (1966).

56b. Mollica, J. A., Jr. and Connors, K. A., J. Am. Chem. Soc., 89, 308 (1967).

57. Rossotti, F. J. C. and Rossotti, H., "The Determination of Stability Constants in Solution," McGraw-Hill Book Co., Inc., N.Y., 1961.

58. Kuntz, I. D., Jr., Gasparro, F. P., Johnston, M. D., Jr. and Taylor, R. P., J. Am. Chem. Soc., 90, 4778 (1968).

59. Utsumi, H., Fujii, K., Irie, H., Funisaki, A. and Nitta, I., Bull. Chem. Soc. Japan, 40, 426 (1967).

60. Leiserowitz, L. and Schmidt, G. M. J., Acta Cryst., 18, 1058 (1965).

61. Sutor, D. J., J. Chem. Soc., 1105 (1963).

62. Guttman, D. and Higuchi, T., J. Am. Pharm. Assoc., Sci. Ed., 46, 4 (1957).

63. Emery, W. O. and Wright, C. D., J. Am. Chem. Soc., 43, 2323 (1921).

64. Tiemann, F. and Herzfeld, H., Ber., 10, 283 (1877).

65. Reiche, F., Ber., 22, 2356 (1889).

66. Tiemann, F. and Herzfeld, H., Ber., 10, 63 (1877).

67. Bender, M. L. and Zerner, B., J. Am. Chem. Soc., 84, 2550 (1962).

68. Buck, J. S. and Heilbron, I. M., J. Chem. Soc., 121, 1100 (1922).

69. Zincke, Th. and Mühlhausen, G., Ber., 36, 134 (1903).

70. Hoeflake, J. M. A., Rec. trav. Chim., 36, 30 (1916).

71. Chattaway, F. D., J. Chem. Soc., 2495 (1931).

72. Irvine, F. M. and Robinson, R., ibid., 2091 (1927).

73. Fischer, F. and Bürgin, A., Pharm. Acta Helv., 31, 518 (1956).

74. Galatis, L. C., J. Am. Chem. Soc., 69, 2062 (1947).

75. Vogel, A. I., "Practical Organic Chemistry," 3rd ed., Wiley and Sons, N.Y., 1956.

76. Zioudrou, C. and Fruton, J. S., J. Am. Chem. Soc., 79, 5951 (1957).

77. Stoermer, R., Grimm, F. and Laage, E., Ber., 50, 959 (1917).

78. Gane's Chemical Works, Inc., British Patent 756,594 (1956).

79. Posner, T., J. Prakt. Chem., 82, 430 (1910).

80. Schwabe, W., Archiv Pharm., 245, 323 (1906).

81. Zelnik, R., Pesson, M. and Polonovski, M., Bull. Soc. Chim., 1773 (1956).

82. Fickett, W., Garner, H. K. and Lucas, H. J., J. Am. Chem. Soc., 73, 5063 (1951).

83. Dewael, A., Bull. Soc. Chim. Belg., 39, 395 (1930).

84. Szendey, G. L., Arch. Pharm., 299, 527 (1966).

85. Schunack, W., Mutschlen, E. and Rochelmeyer, H., Deut. Apotheker-Ztg., 105, 1551 (1965).

86. Alexander, T. G. and Maienthal, M., J. Pharm. Sci., 53, 962 (1964).

87. Bates, R. G., J. Res. Nat. Bur. Stand., 66A, 179 (1962).

88. Bates, R. G. and Bower, V. E., Anal. Chem., 28, 1322 (1956).

89. Boyd, W. C., J. Biol. Chem., 240, 4097 (1965).

90. Guggenheim, E. A., Phil. Mag., 2, 538 (1926).

91. Kramer, P. A. and Connors, K. A., Am. J. Pharm. Educ., 33, 193 (1969).

92. The Ealing Corporation, Cambridge, Mass.

93. Cohen, L. A. and Jones, W. M., J. Am. Chem. Soc., 82, 1907 (1960).

94. Personal communication, Paul A. Kramer.

95. Kirsch, J. F. and Jencks, W. P., J. Am. Chem. Soc., 86, 837 (1964).

96. Jencks, W. P. and Carriuolo, J., ibid., 82, 1778 (1960).

97. Connors, K. A., "A Textbook of Pharmaceutical Analysis," John Wiley and Sons, Inc., N.Y., 1967.

98. Kosower, E. M., "An Introduction to Physical Organic Chemistry," John Wiley and Sons, Inc., N.Y., 1968.

99. Davis, K. M. C. and Symons, M. C. B., J. Chem. Soc., 2079 (1965).

100. Emslie, P. H. and Foster, R., Rec. trav. Chim., 84, 255 (1965).

101. Scott, R., DePalma, D. and Vinogradov, S., J. Phys. Chem., 72, 3192 (1968).

102. Hatton, J. V. and Schneider, W. G., Can. J. Chem., 40, 1285 (1962).

103. Green, R. D. and Martin, J. S., J. Am. Chem. Soc., 90, 3659 (1968).

104. Hata, S. and Tomioka, S., Chem. Pharm. Bull., (Tokyo), 16, 2078 (1968).

105. Hall, A. and Wood, J. L., Spectrochim. Acta, Part A, 24, 1109 (1968).

106. Nakano, M., Ph.D. dissertation, University of Wisconsin, Madison, Wisconsin, 1968.

107. "Handbook of Chemistry and Physics," R. C. Weast and S. M. Selby, eds., The Chemical Rubber Co., Cleveland, Ohio, 1967.

108. Bender, M. L., Chem. Rev., 60, 53 (1960).

109. Personal communication, Martin H. Infeld.

110. Ballinger, P. and Long, F. A., J. Am. Chem. Soc., 82, 795 (1960).

111. Hine, J. and Hine, M., ibid., 74, 5266 (1952).

112. Leffler, J. E. and Grunwald, E., "Rates and Equilibria of Organic Reactions," John Wiley and Sons, Inc., N.Y., 1963.

VI. APPENDIX

A. SINGLE-STEP STABILITY STUDIES

B. RELEASE OF A DRUG FROM A DOSAGE FORM

C. AN IMPROVED METHOD OF PRODUCING FLUORINE 18 FOR USE IN MEDIUM FLUX REACTORS