extracted (Soxhlet) for 12 hr with 300 ml of acetone. The acetone was evaporated and the solid residue slurried and filtered from 75 ml of carbon tetrachloride to yield 7.5 g (55%) of *p,p'*-dinitrobibenzyl.

The reaction was repeated using 27.3 g of potassium metal and 550 ml of *t*-butyl alcohol. 2-Nitropropane (15 ml, 0.5 mole) was added, followed (15 min) by 13.8 g (0.1 mole) of *p*-nitrotoluene in 25 ml of *t*-butyl alcohol. After 35 min *p,p'*-dinitrobibenzyl was isolated, as described previously, in yields of 59 and 57% in two reactions. From the green filtrate from the initial filtration of *p,p'*-dinitrobibenzyl, 2-nitroso-2-nitropropane, mp 71–73°, slowly precipitated. Extraction of the green filtrate with five 100-ml portions of chloroform yielded 4.15 g of a residue not vaporized at 25° and 10 mm. A portion of the residue was dissolved in ethyl acetate and analyzed by gas–liquid partition chromatography. 1-(4-Nitrophenyl)-2-methyl-2-nitropropane was identified with the pure material on a 2-m 3% SE-30 silicone rubber–Chromosorb P column at 172°. The total yield of the coupled product was 0.6–0.7%, based on *p*-nitrotoluene. Extraction of the entire solid residue with carbon tetrachloride left 0.24 g (0.56%, based on 2-nitropropane) of 2,3-dimethyl-2,3-dinitrobutane, mp 212–214°. Repetition of the whole procedure omitting the *p*-nitrotoluene yielded 0.02 g (0.04%) of 2,3-dimethyl-2,3-dinitrobutane. The coupled products formed in the mixed reaction were at least 28.4 mmoles of *p,p'*-dinitrobibenzyl, at least 1.36 mmoles of 2,3-dimethyl-2,3-dinitrobutane, and not more than 0.7 mmole of *p*-NO_2-$C_6H_4CH_2C(CH_3)_2NO_2$.

Modification of Reaction Rates by Complex Formation. II. Inhibition of the Rate of Alkaline Hydrolysis of Methyl *trans*-Cinnamate by Some Heterocyclic Compounds[1]

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Abstract: The rate of alkaline hydrolysis of methyl *trans*-cinnamate is decreased in the presence of several imidazole and purine compounds. This effect is ascribed to formation of one or more complexes between the ester substrate and the heterocyclic ligand, the complexed form of the ester being much less susceptible to attack by hydroxide than is the free ester. Apparent stability constants, based on an assumed 1:1 stoichiometry, were evaluated by the solubility, spectral, and kinetic techniques; these comparative measurements provided information bearing on the actual stoichiometric relationships. Many of the ligands appear to form 1:1 complexes, but some systems are more complicated. The apparent stability constants (at 25° in aqueous solution) ranged from 1.0 M^{-1} for the imidazole complex to 36 M^{-1} for caffeine (which, however, does not form only a 1:1 complex). Xanthines were the strongest complexers of methyl cinnamate, with the purines, as well as simple imidazole compounds, being less effective. Conversion of the neutral theophylline molecule (stability constant 24 M^{-1}) to its anion reduced its effectiveness (anion stability constant 12 M^{-1}); this was attributed to extensive solvation of the anion. Possible complications in catalytic studies may arise when unsuspected complexing occurs to cause concurrent inhibition.

The concept of complex formation as a rate-mediating phenomenon is a familiar one in studies of enzyme-catalyzed reactions, and an adequate understanding of the mechanisms of enzymatic reactions will require that the complex formation aspect be understood far better than it is at present. Both the reactivity and the specificity behavior of enzymes as catalysts appear to be closely related to their capabilities for intermolecular and intramolecular binding interactions. In studies of the high catalytic efficiency of enzymes successful use has been made of relatively simple nonenzymatic model systems as aids in locating and elucidating possible mechanistic pathways. The associated problem of enzyme specificity behavior may similarly be attacked, and this paper presents results of studies planned to provide information on the modification of reaction rates by complex formation in homogeneous, nonenzymatic systems. The point of view tentatively adopted is that specificity patterns may reflect a more active process than simply a suitable juxtaposition of labile and catalytic functions in the enzyme–substrate complex; rather it is supposed that specificity can also be the manifestation of interactions that cause or prevent necessary combinations of groups in space.[1] Thus rate inhibitions caused by complex formation may be as meaningful for this aspect of model studies as are rate enhancements, and the systems to be described here all demonstrate inhibitory effects.

The term "complex" has been assigned many meanings. In this paper it signifies a stoichiometric product formed in a very facile equilibrium process by interaction between two or more species, the product not being susceptible to description in conventional bond symbolism. The existence of such complex species is inferred from nonadditive behavior in the physical and chemical properties of solutions of the interacting species and sometimes from the appearance of insoluble complexes. Hydrogen bonding, charge-transfer processes, dispersion forces, electrostatic interactions, etc., are responsible for the existence of complex species; solvent orientation effects may also be important.

An earlier communication[1] reported that the rate of alkaline hydrolysis of methyl *trans*-cinnamate[2] is decreased in the presence of imidazole. This observation seemed remarkable in view of the well-known catalytic properties of imidazole,[3,4] and the investiga-

(1) For the first paper in this series see K. A. Connors and J. A. Mollica, Jr., *J. Am. Chem. Soc.*, 87, 123 (1965).

(2) Throughout this paper the *trans* isomer is to be understood when methyl cinnamate is specified.

tion has been extended to explore the validity of the complexation hypothesis and the relationship of the structure of the inhibitory agent (which we will call the ligand) to its efficacy in causing a rate decrease. Additional studies aimed at elucidating the relationship of the structure of the substrate (that is, the species whose properties are being measured) to the inhibitory effect are now under way.

Experimental Section

Materials. Methyl *trans*-cinnamate (Matheson Coleman and Bell) was distilled under reduced pressure; bp 91–94° (2–3 mm); mp 33–34° (lit.[5] 33.5–34.5°). The molar absorptivity at 279 mμ (the band maximum) was ϵ_{279} 2.19×10^4 (lit.[6] 2.21×10^4) in water. Its purity was checked by spectrophotometric determination of the cinnamate ion liberated upon alkaline hydrolysis; the ester was found to be at least 99% pure by this criterion. The second-order rate constant for alkaline hydrolysis of methyl cinnamate at 25°, based on hydroxide ion concentration, is $6.18 \pm 0.15 \times 10^{-2}$ M^{-1} sec^{-1} in 0.4% acetonitrile, a value in excellent agreement with the one, $6.04 \pm 0.10 \times 10^{-2}$ M^{-1} sec^{-1}, reported by Bender and Zerner.[5] The second-order constant based on hydroxide activity (from pH measurements) was found to be $8.26 \pm 0.20 \times 10^{-2}$ M^{-1} sec^{-1}, giving an apparent molar activity coefficient for hydroxide ion of 0.75; the ionic strengths employed ranged from 0.1 to 0.3. In these calculations K_w was taken to be 1.00×10^{-14} at 25°.

trans-Cinnamic acid (Matheson) was recrystallized from ethanol–water; mp 133–134° (lit.[6] 134–135°); ϵ_{269} 2.02×10^4 (lit.[6] 2.03×10^4) for cinnamate ion in water.

Imidazole (Aldrich Chemical Co.) was recrystallized three times from benzene using Norit as a decolorizing adsorbent, mp 89–90° (lit.[7] 89–90°). 2-Methylimidazole (Aldrich Chemical Co.) was recrystallized twice from benzene using Norit, mp 144–145° (lit.[7,8] 144°, 140–141°). N-Methylimidazole (K & K Laboratories, Aldrich Chemical Co.) was distilled through a packed column, bp 194° (748 mm) (lit.[9] 195–197° [760 mm]). Benzimidazole (Eastman Kodak White Label) was recrystallized three times from water using Norit, mp 172–173° (lit.[7] 171–173°).

Guanine and purine (Mann Research Laboratories, "Mann Assayed") were used directly. Guanine did not melt up to 360° and purine had mp 216–217° (lit.[10] 215–216°). Uracil (National Biochemical Corp.) was recrystallized from water, mp 334°, turning brown around 300° (lit.[11] 335°, turns brown around 280°).

Caffeine (USP grade) was recrystallized from water, mp 235–236°. The solubility of caffeine in pH 8.5 borate buffer was 0.108 M at 25°, in excellent agreement with the value 0.107 M in water reported by Emery and Wright.[12] Its solubility in pH 8.5 borate buffer containing 1% acetonitrile was 0.113 M. The solubility was determined by measuring the absorbance of a saturated solution appropriately diluted: ϵ_{273} 1.00×10^4.

8-Chlorotheophylline (Aldrich Chemical Co.) was recrystallized from ethanol–water, mp 290–292° dec (lit.[13] 291°). Its purity was checked by potentiometric titration with standard sodium hydroxide; the sample assayed 100.0%. The pK_a, determined potentiometrically in 15% acetonitrile at 25°, was 5.38 (lit.[14] 5.28). 8-Chlorotheophylline appeared to be stable for at least 1 week in 0.07 N NaOH and for 2 months in pH 9 borate buffer, as determined spectrophotometrically.

Theophylline-7-acetic acid (Aldrich Chemical Co.) was recrystallized from 95% ethanol, mp 269–270° (lit.[15] 269–270°). The purity was determined by titration with sodium hydroxide and was found to be 99.5%, based on the monohydrate. The pK_a, determined potentiometrically in 10% acetonitrile, was 3.20. That the sample was the monohydrate was established by weight loss on drying at 150°. It has been reported that degradation of the pyrimidine ring occurs in strong alkali.[16] This was investigated spectrophotometrically, since a marked spectral change should occur upon ring cleavage. The half-life was estimated to be approximately 2 days in 0.16 N NaOH. This decomposition was considered to occur to a negligible extent in the present studies, where the maximum hydroxide ion concentration was 0.04 N and the time involved was a matter of minutes.

Theophylline (Merck & Co., USP grade) was recrystallized from water, mp 270–271°. That the sample was the monohydrate was determined by drying at 150° and by titration in N,N-dimethylformamide with standard sodium methoxide in benzene–methanol as the titrant; thymol blue was the indicator. The solubility of theophylline in pH 6.75 phosphate buffer containing 1% acetonitrile was found to be $3.58 \pm 0.03 \times 10^{-2}$ M by spectrophotometric analysis; ϵ_{272} 1.00×10^4.

Acetonitrile (Eastman Kodak Co., technical grade;[17] Matheson Coleman and Bell, practical grade) was refluxed over phosphorus pentoxide[18] and distilled twice from phosphorus pentoxide; the final distillation was through a packed column, bp 80–81°. Isooctane (2,2,4-trimethylpentane) (Matheson Coleman and Bell, practical grade) was shaken with concentrated sulfuric acid intermittently for at least 2 days, washed, filtered through anhydrous potassium carbonate, and distilled, bp 98–99°. Silicic acid (Mallinckrodt, A.R.) was used directly. Buffer chemicals were of reagent grade. Water was redistilled from alkaline permanganate. Buffers were prepared according to Bates and Bower[19] and Kolthoff.[20]

Apparatus. pH was measured with a Radiometer pH meter Model 25 with scale expander, equipped with a wide pH range glass electrode G202B. The meter–electrode system was standardized against the basic standard buffers recommended by Bates.[21]

Water bath temperatures were maintained to ±0.05° with Sargent Thermonitor electronic relays. Thermometers were checked against a thermometer carrying a National Bureau of Standards certificate.

Spectral measurements were made with a Cary Model 14 recording spectrophotometer, which was fitted with a thermostated cell compartment that maintained temperature constant to ±0.1°. The spectrophotometer absorbance scale was checked against the alkaline chromate standard suggested by Haupt.[22]

Solubility determinations were accomplished with an apparatus capable of rotating ten 15-ml screw-cap vials in a constant temperature water bath. This apparatus was composed essentially of a vertical shaft rotated from the top by an electric motor (whose speed could be varied), and connected through gears at its lower end to a horizontal axle, which was rotated at about 70 rpm. The vials were fastened radially, so that they tumbled end over end.

Procedures. Kinetic Measurements. The procedure employed depended upon the half-life of the reaction. (i) Reactions with half-lives in the range 2–8 min. In a typical experiment, cuvettes with 3.0 ml of buffer containing the ligand were placed in the reference and sample compartments of the spectrophotometer and were allowed to stand for 15 min to reach temperature equilibrium. The spectrophotometer signal was balanced and then 25 μl of acetonitrile was added to the reference cell and 25 μl of ester in acetonitrile solution was added to the sample cell, which was covered with a Teflon stopper. Recording of absorbance (as a function of time at constant wavelength) was usually started within 10 sec of initiation of the reaction. Reactions were followed for about two half-lives, though at least one of the samples containing ligand was observed to about 95% of completion to establish that

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the reaction adhered to apparent first-order kinetics throughout its course. (ii) Reactions with half-lives in the range 8–25 min. The buffer solution (25 ml) containing ligand was equilibrated in a water bath. Then 0.2 ml of ester in acetonitrile solution was added to initiate the reaction. This solution (3 ml) was rapidly transferred to a cell that had been equilibrating in the spectrophotometer cell compartment. Recording was usually started within 50 sec of zero time. (iii) Reactions with half-lives greater than 1 hr. The appropriate solution was prepared in a 100-ml volumetric flask, which was placed in a water bath. Ester (1 ml) in acetonitrile solution was added, and the solution was brought to volume. Samples were withdrawn at suitable intervals for analysis.

The reactions were followed at either 295 or 320 mμ, depending upon the spectral properties of the ligand. For analyses at 295 mμ the initial ester concentration was about 6×10^{-5} *M* and for studies at 320 mμ it was about 7×10^{-4} *M*.

In an additional experiment a set of determinations was performed as follows using caffeine as the ligand. Solutions were prepared in which the initial ester concentration was 5×10^{-4} *M*; 5-ml portions were withdrawn at appropriate time intervals and were extracted with 5.0 ml of isooctane. The isooctane extract (3 ml) was placed on a partition chromatographic column (internal phase 20 g of silicic acid and 20 ml of pH 7.4 phosphate buffer; external phase isooctane). The eluate (50 ml) was collected, and the absorbance of the unreacted ester was measured at its absorption maximum.

Stock solutions of the ligand were prepared by adding a weighed amount to a volumetric flask; if the ligand was ionizable at the pH of the studies, an equivalent amount of 1 *N* NaOH was added. A buffer solution and sufficient KCl to adjust the ionic strength were added. This solution was brought to temperature and its pH was measured. The buffer stock solution was adjusted to the same pH by adding small volumes of hydrochloric acid or sodium hydroxide solution. Then solutions with different concentrations of ligand, but identical pH, could be prepared by dilution of the ligand stock solution with the buffer stock solution.

Rate constants were obtained from plots of log $(A_t - A_\infty$ against time or by the method of Guggenheim.[23]

Solubility Measurements. Methyl cinnamate in excess of its solubility (which is about 2.2×10^{-3} *M* at 25° in 1% acetonitrile) was added (in 0.1 ml of acetonitrile solution) to 10.0 ml of buffer solution containing varying concentrations of ligand. The ligand concentrations were obtained by dilution of a stock solution (for those concentrations below its solubility) or by adding accurately weighed amounts (for concentrations in excess of its normal solubility). The vials were closed and rotated in a constant temperature bath for at least 24 hr. A portion of the supernatant solution was withdrawn from each vial by pipet, around the tip of which had been wrapped glass wool to act as a filter. For those ligands without an absorption band in the analytical wavelength region, the sample was diluted with water and the concentration of methyl cinnamate determined spectrophotometrically. Samples containing absorbing ligands that were insoluble in isooctane were extracted with this solvent; the organic extract was diluted and examined spectrophotometrically. When caffeine was the ligand a partition chromatographic separation (as described earlier) was carried out.

The absorption spectra after equilibration and dilution were all identical with that of pure methyl cinnamate, indicating that any changes in solubility were not caused by irreversible chemical reaction between the ester and the ligands.

Apparent stability constants were calculated from plots of total apparent molar solubility of substrate against total molar concentration of ligand.[24,25]

Spectral Studies. Stability constants were also evaluated from spectroscopic measurements. A typical experiment was conducted as follows. Two 25-ml volumetric flasks, each containing the same amount of ligand in a buffer, were equilibrated. To one flask was added 0.2 ml of pure acetonitrile, to the other 0.2 ml of ester in acetonitrile. The former solution (3 ml) was placed in the reference cell and 3 ml of the latter in the sample cell; these were brought to temperature equilibrium in the spectrophotometer. The absorption was then measured, usually at 320 mμ. The solutions containing buffer and ligand were usually filtered through a sintered glass funnel.

Other procedures were sometimes used. The appropriate solution (3 ml) was placed in each of two 1-cm cuvettes, which were equilibrated in the spectrophotometer. After the signal was balanced, 25 μl of acetonitrile was added to the reference cell and 25 μl of ester in acetonitrile to the sample cell, and the absorbance was recorded. It was also possible to obtain such data from kinetic studies by extrapolation to zero time on plots of log $(A_t - A_\infty)$ against time.

For some systems a new method was employed in which a saturated solution of substrate (ester) was placed in the reference cell and a solution containing a known concentration of ligand, also saturated with respect to substrate, was in the sample cell. (These solutions were obtained from the solubility studies.) Spectra were determined over the wavelength range where absorption by ligand was negligible. Because both solutions are saturated with respect to uncomplexed substrate, the difference spectrum observed must be due to the complex or complexes present in the sample solution.

Partition Studies. A method similar to that of Guttman and Higuchi[26] was employed to determine the concentration of monomeric caffeine at any given total caffeine concentration. Volumes of a 0.09 *M* caffeine solution in pH 9.2 borate buffer were measured by buret into 15-ml vials; buffer solution was added, and the vials were stoppered with Teflon-lined screw caps, and rotated for 10 hr at 25.0° with the solubility apparatus described earlier. The organic layer was assayed spectrophotometrically.

Treatment of Data. In evaluating complex stability constants from solubility, spectral, and kinetic measurements, a 1:1 stoichiometry between substrate and ligand is conventionally assumed in the absence of evidence implicating other stoichiometries. Often the apparent 1:1 stability constants (here symbolized K_{11}') as evaluated for a single system by the three methods are quite different. Probably the usual reason for this disagreement is failure of the assumption that only a 1:1 complex is present. A recent analysis[25] of this problem indicates that comparative studies of complex formation using several techniques may be helpful in elucidating the stoichiometries of the complexes. A brief summary of this analysis will be useful in later sections.

The operational definitions of the apparent 1:1 stability constants may be summarized as follows.

Solubility. Plot total apparent molar solubility of substrate $[S_t]$ against total molar concentration of ligand $[L_t]$; then

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

Spectral. Plot $b/\Delta A$ (where b is path length and ΔA is absorbance difference between solutions with and without ligand) against $1/[L_t]$; then

$$K_{11}' = \text{intercept/slope} \quad (2)$$

Kinetic. Plot $1/(k_S - k_S')$ (where k_S and k_S' are rate constants in the absence and presence of ligand) against $1/[L_t]$; then

$$K_{11}' = \text{intercept/slope} \quad (3)$$

Several criteria have been suggested[25] to help in establishing stoichiometries and stability constants.

(a) Relative values of K_{11}' by the solubility, spectral, and kinetic techniques. Analysis of k_{11}' in terms of the actual constants of the system, for several stoichiometries and for multiple complexes, provides a partial means for classifying the system stoichiometry. Thus if all three methods yield the same numerical value, the system probably contains only 1:1 complexes.

(b) Dependence of K_{11}' on initial total substrate concentration by the spectral and kinetic techniques. When a complex S_mL_n is present for which m is greater than one, K_{11}' by these two techniques will be a function of substrate concentration.

(c) Dependence of K_{11}' on ligand concentration by the solubility technique. If a positive curvature is observed in the solubility diagram at least one complex is present of the form SL_n where n is greater than one. Negative curvature may indicate dimerization of the ligand. Although nonlinear kinetic and spectral plots are theoretically to be expected when stoichiometries other than 1:1 are present, it is known[27] that the deviation from linearity may not be observable.

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(d) Dependence of k_S' on time. When a complex is present with two or more S molecules per complex molecule, the apparent rate constant may vary with time.

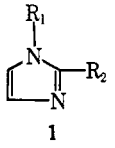
(e) Dependence of K_{11}' on wavelength in the spectral technique. If K_{11}' varies with the wavelength of observation, at least one higher order complex is present.[25,27]

(f) Independent evidence relating to stoichiometry and stability. Among the types of evidence are liquid–liquid partition studies and analysis of isolable complexes.

These criteria have been applied in the present study, and the results will be discussed in these terms.

Results

Imidazole. In 1957 Bender[28] and Bruice[7] and their co-workers reported that the hydrolysis of *p*-nitrophenyl acetate is catalyzed by imidazole **1a**. Since that time many studies of the catalytic properties of this substance have been made.[3,4]



a, $R_1=R_2=H$
b, $R_1=H$; $R_2=CH_3$
c, $R_1=CH_3$; $R_2=H$

1

The recent observation has been reported that imidazole *decreases* the rate of hydrolysis of methyl cinnamate.[1] On the assumption that inhibition is caused by the formation of a complex between substrate (methyl cinnamate) and ligand (imidazole), the complex being less reactive toward hydroxide ion than is the free substrate, it is possible to analyze the rate data as described in the preceding section; the value $K_{11}' = 0.9 \pm 0.1\ M^{-1}$ was obtained, and the presumed 1:1 complex appeared to be essentially unreactive. The solubility $K_{11}' = 1.0 \pm 0.1\ M^{-1}$. A small bathochromic shift in the spectrum of the ester was noted in the presence of imidazole, but a spectral constant could not be estimated with accuracy.

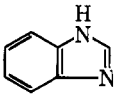
Although the inhibitory effect of imidazole is small, it appears to be greater than can be ascribed to a general solvent effect. For example, Kirsch and Jencks[29] have determined activity coefficients for trifluoroethyl acetate and ethyl acetate in 1 *M* imidazole (95% free base); they found that the ratio of solubilities in 1 *M* imidazole and in water was 1.12 for the trifluoroethyl ester and 1.37 for the ethyl ester. In comparison, this ratio for methyl cinnamate is 2.50. Another comparison can be made by studying the inhibitory effect of a compound believed to act only by its nonspecific effect upon solvent properties; thus 1 *M* acetonitrile produced only an 8% decrease in rate, while 0.79 *M* imidazole produced a 43% decrease.

The spectral shift cannot be caused by the formation of an acylimidazole, since the acylimidazole formed in this system would be cinnamoylimidazole, which absorbs powerfully at 310 mμ; moreover, if it were formed, catalysis would be observed because the alkaline rate constant for cinnamoylimidazole is more than a thousandfold greater than is that of methyl cinnamate.[30]

2-Methylimidazole. Kinetic and solubility studies with this ligand (**1b**) revealed behavior similar to that observed with imidazole: $K_{11}' = 1.2\ M^{-1}$ by the solubility method and 1.0 M^{-1} from the kinetic data.

N-Methylimidazole. This compound (**1c**) caused a rate inhibition, but the effect was qualitatively different from that observed with imidazole and 2-methylimidazole, both of which lead to hyperbolic plots of apparent rate constant k_S' against $[L_t]$, indicating an equilibrium process. In contrast, the same plot with N-methylimidazole is linear to 0.8 *M* ligand concentration; at this concentration a rate decrease of about 50% is observed. The linearity of the relationship suggests the operation of some mechanism other than an equilibrium process, though the possibility exists that a combination of two effects could lead to the observed plot. The magnitude of the inhibition by N-methylimidazole is about the same as that caused by imidazole and 2-methylimidazole.

Benzimidazole. In concentrations up to 0.03 *M*, benzimidazole (**2**) had no significant effect on the solubility or the spectrum of methyl cinnamate. The low



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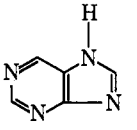
solubility of this ligand prevented studies at higher concentrations. By taking into account the expected uncertainty of the measurements, it is estimated that the stability constant for this system must be less than 3 M^{-1}. Kinetic measurements showed a slight inhibiting effect; these studies were carried out at pH 12.8, so a large fraction of the ligand was in its anionic form (pK_a[31] = 12.57) and a maximum total concentration of 0.06 *M* benzimidazole was employed. The estimated stability constant is less than 1 M^{-1}.

Purine. The methyl cinnamate–purine (**3**) system was studied kinetically, the maximum concentration of ligand being about 0.1 *M*. At pH 12.77, in 0.83% acetonitrile and at ionic strength 0.3, the stability constant K_{11}' was found to be 2.3 M^{-1}. The reactivity of the complex appears to be negligible. Under the conditions of the experiment purine was in its anionic form.



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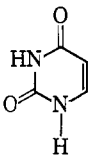

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Uracil. Uracil (**4**) was the only ligand employed that does not contain the imidazole ring; it was included in the study because it represents the six-membered ring of the xanthine structure. This compound caused no significant change in the spectrum of methyl cinnamate. The hydrolytic rate of the ester was decreased about 6% in the presence of 0.066 *M* uracil. The apparent stability constant (from the rate measurements) is less than 1 M^{-1}. The molecule was in the anionic form in these studies.

8-Chlorotheophylline. The xanthines are purine derivatives with the general structure **5**. 8-Chlorotheophylline (**5a**) was studied as the ligand by the solubility,

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(29) J. F. Kirsch and W. P. Jencks, *ibid.*, **86**, 837 (1964).

(30) G. R. Schonbaum, B. Zerner, and M. L. Bender, *J. Biol. Chem.*, **236**, 2930 (1961).

(31) H. Walba and R. W. Isensee, *J. Am. Chem. Soc.*, 77, 5488 (1955).

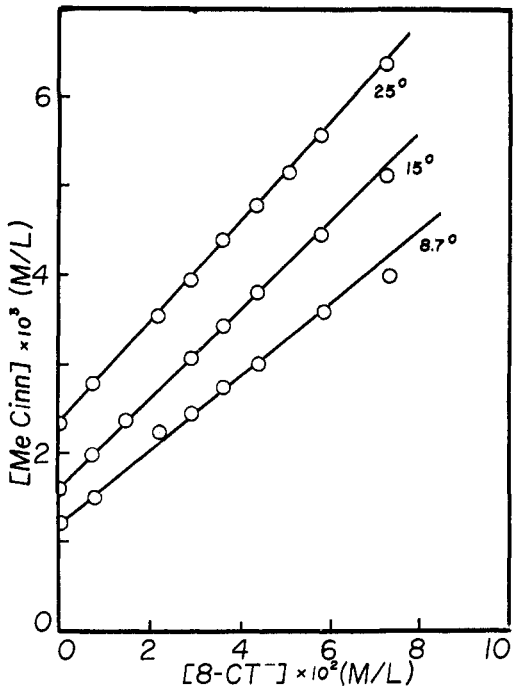
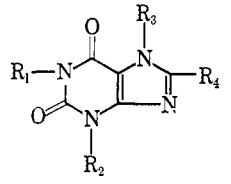


Figure 1. The apparent solubility of methyl cinnamate as a function of 8-chlorotheophylline concentration.

spectral, and kinetic techniques. Because the pK_a of this compound is 5.3, it was present as the anion in these studies, and this form is to be understood when 8-chlorotheophylline is specified.



a, $R_1=R_2=CH_3$; $R_3=H$; $R_4=Cl$
b, $R_1=R_2=CH_3$; $R_3=CH_2COOH$; $R_4=H$
c, $R_1=R_2=CH_3$; $R_3=R_4=H$
d, $R_1=R_2=R_3=CH_3$; $R_4=H$

5

Solubility studies were conducted at three temperatures. As shown in Figure 1, the phase diagrams were linear. The K_{11}' values evaluated according to eq 1 were 26 M^{-1} (25.0°), 32 M^{-1} (15.0°), and 36 M^{-1} (8.7°). From these quantities the thermodynamic values $\Delta H° = -3.4$ kcal/mole and $\Delta S° = -4.8$ eu were calculated for the complex formation equilibrium over the temperature range studied.

A marked change in the spectrum of methyl cinnamate was observed in the presence of 8-chlorotheophylline. Figure 2 shows plots for spectral data at two substrate concentrations; K_{11}' evaluated by eq 2 was 26 M^{-1} for both ester concentrations at 25.0°. In another experiment at an ester concentration of 7.3×10^{-4} M, K_{11}' was 23 M^{-1} at 25° and 18 M^{-1} at 35°.

The rate of hydrolysis of methyl cinnamate was markedly decreased by 8-chlorotheophylline. The linear plot is shown in Figure 3, and K_{11}' calculated from eq 3 was 22 M^{-1} at 25.0°. From the intercept[25] the complex was estimated to be about 9% as reactive as the free ester.

The estimated uncertainty in the stability constants is ±10%, and it is concluded that there are no significant differences among the constants evaluated at 25° by the three methods; the value 24 ± 2 M^{-1} can be assigned to the system at this temperature. The agreement of the constants is evidence that the complex (or complexes) possesses a 1:1 stoichiometry.

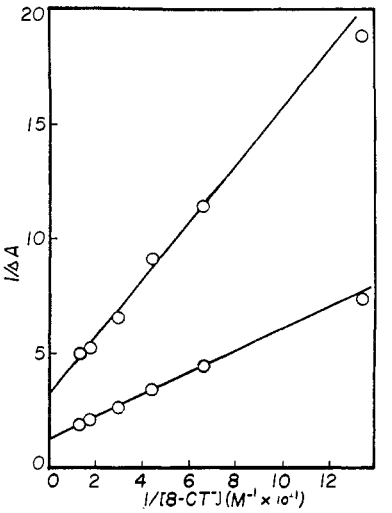


Figure 2. Plots of spectral data for the 8-chlorotheophylline system (pH 8.5 borate buffer, 1% acetonitrile, 25.0°, ionic strength 0.1). Lower curve, 6.16×10^{-4} M ester; upper curve, 2.38×10^{-4} M ester.

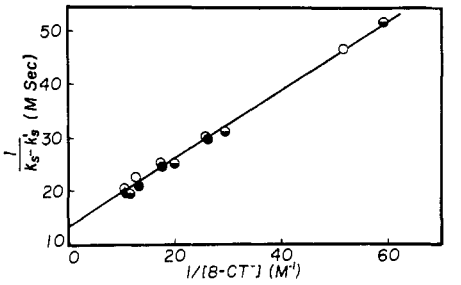


Figure 3. Plot of kinetic data for the 8-chlorotheophylline system at 25.0° in 1% acetonitrile: ○, 0.1 N NaOH; ●, pH 12.57 hydroxide-chloride; ◒, pH 10.04 borate buffer.

During the kinetic study it was observed that absorbance values at infinite time did not all approach the same value. This was interpreted as the result of an interaction between the product cinnamate ion and the ligand; the spectral K_{11}' was 6.8 M^{-1} at 25° for this interaction. This is an interesting observation because it indicates that two relatively small ions of the same charge type can interact significantly.

Theophylline-7-acetic Acid. This compound (**5b**) exists as the anion (pK_a = 3.20) at all pH's used in the present study. The charge is localized at the carboxylate group (unlike the charge on theophylline and 8-chlorotheophylline anions, which can be distributed into the ring systems). The solubility K_{11}' was 19 M^{-1}, the kinetic K_{11}' was 14 M^{-1}, and the spectral technique yielded stability constants of 12 and 10 M^{-1} at substrate concentrations of 2.9×10^{-4} M and 6.4×10^{-4} M, respectively. It seems probable that one or more complexes are present with higher order stoichiometries.

Theophylline. Theophylline (**5c**) ionizes in a convenient pH range (pK_a = 8.7), allowing study of its interaction in both forms. (Hereafter the anionic form will be referred to as theophyllinate.)

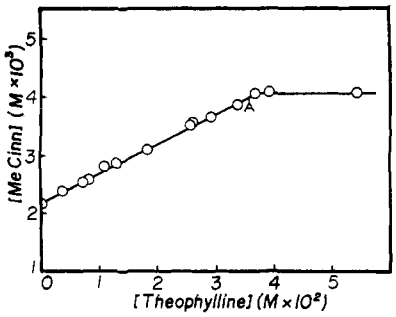


Figure 4. The apparent solubility of methyl cinnamate in the presence of theophylline in pH 6.57 phosphate buffer containing 1% acetonitrile; ionic strength 0.3, temperature 25.0°.

The solubility isotherm for theophylline is shown in Figure 4; $K_{11}' = 25\ M^{-1}$ from this plot. The plateau region is attributed to the appearance of solid theophylline. A stoichiometric ratio can be roughly estimated from the diagram.[24] Point A represents the normal solubility of theophylline ($3.58 \times 10^{-2}\ M$). The discontinuity is estimated to occur at a total theophylline concentration of $3.76 \times 10^{-2}\ M$, representing an increase of $0.18 \times 10^{-2}\ M$. The solubility increase for methyl cinnamate is given by the vertical distance from the intercept to the solubility at the break point; this is $0.19 \times 10^{-2}\ M$. The stoichiometric ratio is therefore calculated to be 1:1.

K_{11}' was found to be $22\ M^{-1}$ for theophylline when evaluated by the spectral technique (same conditions as for the solubility measurements).

Theophylline, unlike many other purines and xanthines, appears not to dimerize in aqueous solution.[26] It is probable that the theophylline–methyl cinnamate system can be described in terms of a 1:1 complex.

Theophyllinate was investigated by the spectral and kinetic methods. The spectral K_{11}' was $13\ M^{-1}$ and the kinetic K_{11}' was $11\ M^{-1}$, the estimated uncertainty being about 10% in both values. The complex appears to be essentially unreactive. The kinetic data are shown in Figure 5, which shows clearly the effect of theophyllinate on the rate constant (slope) and on the spectrum (intercept); the same initial ester concentration was employed in all of these experiments, and the ligand absorption was negligible at the analytical wavelength.

A stability constant of $4.5\ M^{-1}$ (probable error about 25%) was evaluated for the interaction between theophyllinate and cinnamate ion.

Caffeine. Since 1887, when caffeine (**5d**) was postulated by Daudt[32] to form "molecular compounds" with salicylate and benzoate, many workers have studied the interaction tendencies of this substance. Its choice in the present study was based partly upon the presence of pertinent data in the literature, but also upon the interesting contrast with theophylline, since caffeine can neither ionize nor act as a hydrogen-bonding donor. It was recognized that caffeine is capable of self-aggregation,[12,26] and that it therefore might not yield a simple system for analysis.

The solubility isotherm appears to be reasonably linear and gives a K_{11}' of $36\ M^{-1}$, but careful inspection reveals that it is essentially linear only up to about 0.08 M total caffeine, beyond which a slight positive

(32) C. Daudt, *Pharm. Ztg.*, **32**, 376 (1887).

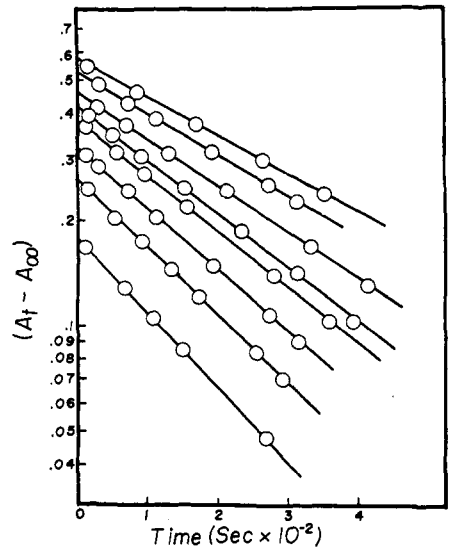


Figure 5. First-order plots for the alkaline hydrolysis of methyl cinnamate in the presence of theophyllinate; temperature 25.0°, pH 12.77, 0.83% acetonitrile, ionic strength 0.3. Theophyllinate concentration ($M \times 10^2$), bottom to top: 0.00, 0.96, 1.92, 2.88, 3.85, 5.77, 7.70, 9.62.

curvature is observed. Analysis of the discontinuity produced by the appearance of a solid phase of caffeine gives an apparent stoichiometry of 1:2.4 (ester:caffeine). This result, taken with the positive curvature, indicates that multiple complexes are present. Similar observations have been made on other caffeine systems.[33]

A complicating factor is the presence of aggregates of caffeine molecules.[26] A partition study was performed to estimate the concentration of monomeric caffeine as a function of total caffeine. The assumption was made that only the monomer partitions into the organic phase. This assumption will be valid if the dimerization constant in the organic phase is less than 10^3, since the concentration in this phase was in the range 10^{-5} to $10^{-4}\ M$. The limiting partition coefficient is defined $K_p = [\text{monomer}]_{aq}/[C]_{org}$, and the apparent partition coefficient is $K_p' = [C]_{aq}/[C]_{org}$; these can be combined to give $[\text{monomer}]_{aq} = K_p[C]_{aq}/K_p'$, which permits a curve to be constructed relating concentration of monomeric caffeine to total caffeine concentration.[34] K_p has the value 215 for the system pH 9.2 borate buffer–isooctane at 25°. The caffeine dimerization constant was estimated to be $19\ M^{-1}$, though the indicated presence of higher aggregates limits the accuracy of this result.

The solubility data were then replotted against caffeine monomer (rather than total) concentration, and the resulting curve was analyzed in terms of 1:1 and 1:2 complexes.[24] (At high caffeine concentrations species containing more than two caffeine molecules are probably present.) The formation of the methyl cinnamate–caffeine complexes could occur by a stepwise buildup of simpler molecules reacting with

(33) (a) L. Lachman, L. J. Ravin, and T. Higuchi, *J. Am. Pharm. Assoc.*, **45**, 290 (1956); (b) L. Lachman and T. Higuchi, *ibid.*, **46**, 32 (1957); (c) M. Donbrow and Z. A. Jan, *J. Chem. Soc.*, 3845 (1963); (d) M. Donbrow and Z. A. Han, *J. Pharm. Pharmacol., Suppl.*, 17, 129S (1965).

(34) J. A. Mollica, Jr., Ph.D. Dissertation, University of Wisconsin, Madison, Wis., 1966.

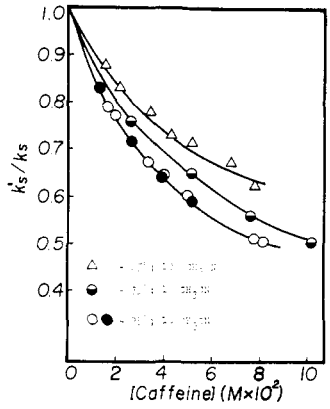


Figure 6. Relative rates of hydrolysis of methyl cinnamate in the presence of caffeine.

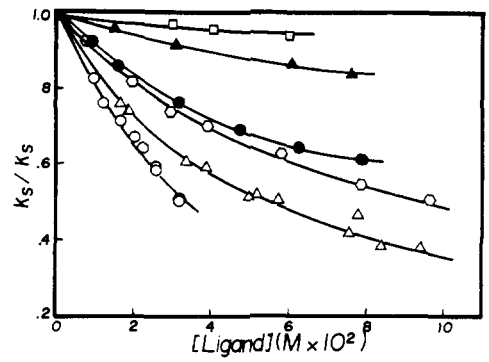


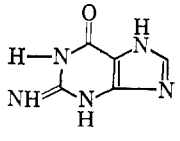
Figure 7. Relative rates of alkaline hydrolysis of methyl cinnamate in the presence of some purines at 25°. Ligands from top to bottom: guanine, purine, theophylline-7-acetate, theophyllinate, 8-chlorotheophyllinate, caffeine (monomer).

monomer: S + L = SL, SL + L = SL_2, SL_{n-1} + L = SL_n; or by combination of substrate with the appropriate aggregate form: S + L = SL, S + L_2 = SL_2, S + L_n = SL_n. The two processes are mathematically equivalent, though the numerical values of constants obtained will depend upon which formulation is chosen. The values obtained in this analysis were K_{11} = 28 M^{-1} and $K_{(12)}$ = 55 M^{-1}, where K_{11} = [SL]/[S][L] and $K_{(12)}$ = [SL_2]/[SL][L].

Caffeine exerted a pronounced effect on the rate of hydrolysis of methyl cinnamate (Figure 6). The analysis of these data suggested that the complexes may be slightly reactive, but the system is too complicated to allow the apparent reactivity to be assigned to a specific complex without introducing arbitrary assumptions; K_{11}' was 18 M^{-1} at 25°. Spectral studies yielded the usual linear plots, but in view of the independent evidence that the system is not a simple one no convenient analysis could be performed, although K_{11}' was calculated to be 18 M^{-1}. The caffeine–methyl cinnamate system illustrates that agreement of K_{11}' values from spectral and kinetic measurements is not sufficient evidence that only a 1:1 complex is present. This system is probably best described, at low caffeine concentrations, as including the complex species SL, SL_2, and L_2.

The extent of interaction in 10% acetonitrile, as observed spectrally and kinetically, appears to be less than in 1% acetonitrile.

Guanine. Guanine (**6**) possesses an imino group rather than a carbonyl in the 2 position. This compound (the anion form was actually present in solution) had no significant effect on the spectrum of methyl cinnamate, and was no more effective than was imidazole in decreasing the rate of hydrolysis. K_{11}'



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from kinetic data was less than 1 M^{-1}. The electronic configuration of the guanine anion is probably markedly different from that of other anions used in this study.

Discussion

Validity of the Complexation Hypothesis. The inhibition of the velocity of alkaline hydrolysis of methyl cinnamate that is observed in the presence of these heterocyclic compounds might be accounted for by either of these hypotheses. (i) The effect may be a general solvent effect; that is, the inhibition is ascribed to a change in the activity of the substrate by an alteration in the solvent's properties upon addition of ligand. This has been referred to as the activity coefficient effect. (ii) A specific phenomenon may be invoked, the hypothesis being that the observed effects are dependent upon interaction between substrate and ligand to form additional species, with altered properties. These new species may be called complexes.

A distinction between the two hypotheses may seem somewhat arbitrary since all deviations from expected behavior could, in principle at least, be described in terms of changes in activity coefficients. The deciding factor is usually the magnitude of the effects observed at given ligand concentration, since many of the techniques used to detect complexes are similar to those used to measure activity coefficients. For example, the solubility method used here is the same as a classical method for determining activity coefficients. However, the size of the observed effects in the present study would appear to rule out a general solvent effect as the sole factor. Some of the data supporting this conclusion have been presented in the Results section, where it is pointed out that even imidazole, one of the least effective of the ligands studied, exerted an effect that could not easily be ascribed to its alteration of solvent properties. It should be pointed out, however, that even when a specific effect appears to be operative, the general solvent effect must also be present, though it is probably often of little importance.

The magnitude of the inhibitory action is not its only aspect that supports the suggestion of specific effects. Table I and Figure 7, which summarize many of the results of this paper, demonstrate the remarkable sensitivity of the effects to the molecular structure of the ligand. Such specificity of action cannot be reconciled with general alterations in the character of the solvent.[35]

(35) A tactical reason for tentatively adopting the hypothesis of a specific interaction is that it is a more fruitful source of ideas than is the activity coefficient hypothesis.

Table I. Apparent 1:1 Stability Constants with Methyl Cinnamate at 25°[a]

Ligand	K_{11}', M^{-1} Solubility	Spectral	Kinetic
Imidazole	1.0	...	0.9
2-Methylimidazole	1.2	...	1.0
Benzimidazole	<3	<3	<1[b]
Purine (anion)	...	...	2.3
Uracil (anion)	...	...	<1
8-Chlorotheophylline (anion)	26	25	22
Theophylline-7-acetic acid (anion)	19	11	14
Theophylline	25	22	...
Theophylline (anion)	...	13	11
Caffeine	36	18	18
Guanine (anion)	...	...	<1

[a] In 1% or 0.83% acetonitrile. [b] Partly in anion form.

It may be supposed that investigation of alleged complex formation by techniques independent of rate measurements might provide unambiguous evidence of the presence of complexes, but, as we have noted above, all such evidence can equally well be interpreted in terms of activity coefficients. Some authors have taken agreement of apparent stability constants evaluated by two methods as evidence that complex formation is indeed responsible for the rate decreases, but it is to be noted that when the estimated constants do not agree, the disagreement is never taken as evidence for the nonintervention of complexes. This attitude does in fact appear to be justified, for it seems probable that few complexing systems will be so simple as to yield identical K_{11}' values by several techniques. The usual reason for discrepancy is the existence of multiple complexes and stoichiometries other than 1:1. The existence of complexes having been recognized, comparative examination by several techniques provides a powerful means for their study,[25] but by themselves the calculated stability constants can neither confirm nor deny the complexation hypothesis.

A further aspect of the discrepancy among independent estimates of the stability constant should be considered. For some reported systems the spectrally measured K_{11}' is considerably smaller than the constant evaluated by other techniques, and some authors have suggested that a smaller spectral K_{11}' implies that only the charge-transfer portion of the interaction forces is being observed by this technique, while other, presumably less selective, techniques observe all of the forces, and therefore produce a larger constant. The error in this argument has been pointed out by several workers.[25,36] If only 1:1 complexes are present, the same value of the stability constant will be provided by every valid experimental technique, no matter what the distribution of forces responsible for maintaining the complexes; this is because all such methods measure activities or concentrations, not forces. If higher stoichiometries are present, different techniques may yield different constants, but the values are not determined by the relative importance of the forces of interaction.

(36) E. Grunwald and J. E. Leffler, quoted by S. E. Ross, M. M. Labes, and M. Schwarz, *J. Am. Chem. Soc.*, **78**, 343 (1956); L. E. Orgel and R. S. Mulliken, *ibid.*, **79**, 4839 (1957).

Many rate inhibitions have been attributed to complex formation, and though other, less specific, effects may sometimes be responsible, much evidence has been presented that complex formation is an important rate-mediating process in nonenzymatic systems. Ross and co-workers showed that hexamethylbenzene inhibits the reaction of triethylamine with picryl chloride,[37] and that aniline inhibits its own reaction with 2,4-dinitrochlorobenzene.[38] Caffeine is a very powerful inhibitor of the coupling of β-naphthol and *p*-diazobenzenesulfonic acid.[39] The inhibition of hydrolysis of some benzoate esters has been attributed to complexing.[33a,b,40-42] Guttman has shown that the rate of alkaline hydrolysis of riboflavin and of related isoalloxazine compounds is decreased by xanthines[14,43] and by borate.[44] A polyanionic ligand inhibited the hydrolysis of a cationic ester.[45] 3,5-Dinitrobenzoate functioned as an inhibitor of the hydrolysis of some carboxylic acid derivatives.[46] Other examples have been reported.[47,48] Thus the general concept of rate decrease by complexation is well accepted, but it is still necessary to examine each fresh instance of inhibitory behavior critically. Person's criterion[49] concerning the significance of a spectroscopically evaluated stability constant is useful in deciding if the evidence is consistent with the assumption of complex formation. In the present study it appears that the formation of complexes of reduced reactivity can reasonably account for the observed phenomena.

It may be noted that an alternate mechanism may be postulated for some of the ligands, especially the simple imidazoles. If a tetrahedral intermediate is formed from nucleophilic attack of the imidazole on the ester, and if the reverse reaction is much more rapid than is conversion to the acylimidazole, an apparent equilibrium situation is created, with the new species being the tetrahedral intermediate, which effectively removes part of the substrate from its reactive form. This hypothesis requires that methoxide be such a poor leaving group that essentially no breakdown to the acylimidazole takes place, and a calculation with the experimentally measured equilibrium constant of 1 M^{-1} shows that when the imidazole concentration is 0.5 M, one-third of the substrate is present as the tetrahedral intermediate. This seems very improbable,[3] and the complexation hypothesis is preferred. The behavior of N-methylimidazole is vexing, and no satisfactory explanation can be given at this time.

Structural Effects on Complex Stability and Reactivity.[50] It is not yet possible to specify with assurance

(37) S. D. Ross, M. Bassin, M. Finkelstein, and W. A. Leach, *ibid.*, 76, 69 (1954).
(38) S. D. Ross and I. Kuntz, *ibid.*, 76, 3000 (1954).
(39) J. Th. G. Overbeek, C. L. J. Vink, and H. Deenstra, *Rec. Trav. Chim.*, **74**, 69 (1955).
(40) T. Higuchi and L. Lachman, *J. Am. Pharm. Assoc.*, **4**, 521 (1955); L. Lachman, D. Guttman, and T. Higuchi, *ibid.*, **46**, 36 (1957).
(41) J. L. Lach and W. A. Pauli, *Drug Std.*, **27**, 104 (1959).
(42) J. L. Lach and T. Chin, *J. Pharm. Sci.*, **53**, 924 (1964).
(43) D. E. Guttman, *ibid.*, **51**, 1162 (1962).
(44) D. A. Wadke and D. E. Guttman, *ibid.*, **53**, 1073 (1964).
(45) H. Morawetz and J. A. Shafer, *Biopolymers*, **1**, 71 (1963).
(46) F. M. Menger and M. L. Bender, *J. Am. Chem. Soc.*, **88**, 131 (1966).
(47) A. Bruylants and J. B. Nagy, *Bull. Soc. Chim. Belges*, **75**, 246 (1966).
(48) R. L. Letsinger and T. E. Wagner, *J. Am. Chem. Soc.*, **88**, 2062 (1966).
(49) W. B. Person, *ibid.*, **87**, 167 (1965).
(50) The conventional meaning of complex *stability*, that is, equi-

the structures of nonisolable complexes such as the ones detected in the present investigation. One of the best ways to develop inferences concerning complex structures is to compare complex stabilities within a series of ligands with a common substrate, as in this work, or a series of substrates with one ligand. Such comparisons have led to correlations of stability with other structural parameters.[24,51]

Another approach, which has not yet been exploited, though some attention has been given to it,[46] is to use complex reactivity, measured in the kinetic approach to complexation studies, as a probe in making structural inferences. Most of the complexes in the present study appeared to be essentially unreactive toward hydroxide ion; this is interpreted to mean that the complexes possess a reactivity certainly not greater than 5% that of the uncomplexed ester. This profound alteration in the susceptibility of the substrate (which is still chemically intact) must be the result of either steric or electronic effects imposed by the stereochemistry of the complex. One possibility emerges for ligands that carry a negative charge; then it seems that electrostatic repulsion of the attacking hydroxide ion might account for a diminished reactivity. However, the neutral ligands imidazole and 2-methylimidazole formed unreactive complexes, while the anion of 8-chlorotheophylline formed a complex with slight reactivity. Menger and Bender,[46] moreover, have shown that when the attacking agent is neutral rather than negatively charged, the efficacy of inhibition by an anionic ligand is not reduced. In complexes of the types being considered, therefore, decrease in reactivity cannot be simply ascribed to electrostatic repulsion.

Steric hindrance to the attacking agent is another possible mode of rate diminution in the complex. When the substrate is a carboxylic acid derivative, and a 1:1 complex is formed, then steric interference by itself cannot account for a complete loss of reactivity. This conclusion follows from the concept of perpendicular attack at carbonyl carbon by an incoming nucleophile;[3] the ligand in the complex can shield only one side of the carbonyl group from attack, so the steric effect can only cause a reduction of 50% in reactivity relative to the uncomplexed substrate. But it must be remembered that if the ligand interacts closely enough with the ester group to protect it sterically, it must also exert a powerful electronic effect, perturbing the orbitals of the substrate and possibly effecting a further decrease in reactivity.

Perturbation of the electronic configuration of the substrate by the interacting ligand could decrease reaction rate by stabilizing the initial state relative to the transition state of the reaction. Whether a pure electronic perturbation (in the absence of any steric effect by the ligand) could account for a nearly total loss of reactivity in the substrate has not yet been satisfactorily established; polar effects by substituents can be of this intensity, but in complex formation the substrate molecule retains its integrity, and electronic alterations, though perhaps profound, probably are not as extensive as in compound formation.

Involvement by the solvent may also be a factor. Both the initial and the transition states may be stabilized by solvation, and the relative extents of such stabilization may be altered by complexation.[46]

Many purines possess essentially planar ring systems,[52] and the ligands used here are probably planar, as is the substrate molecule, which is capable of resonance throughout its length. Electron distributions of some of these ligands have been calculated.[53] With such information and the concepts outlined in preceding paragraphs, models and scale drawings can lead to tentative complex stereochemistries that are at least consistent with experimental observations. For example, in the imidazole–methyl cinnamate complex four of the atoms in the five-membered imidazole ring may lie directly over the three side-chain carbons and one of the oxygens, these four atoms (in the *trans* isomer) having about the correct geometry for this superposition. This stereochemistry would lead to marked steric and electronic effects that might well reduce reactivity. 2-Methylimidazole should be equally effective in complex formation on this basis, for the methyl group would not interfere with close approach of the complexing species.

If the five-membered ring of the xanthine derivatives is assumed to interact in some arrangement directly with the ester group (perhaps as described for imidazole) it is found that the six-membered ring does not fall directly over the phenyl ring of the substrate, but instead involves one or the other edge of the ring. At this stage of investigation, these speculations do not lead to testable quantitative estimates.

Some of the results collected in Table I may now be considered. The superiority of the xanthines in conferring stability is immediately evident. This is observed for both the neutral and anionic forms. The presence of a six-membered ring fused to the imidazole nucleus is clearly not a sufficient condition for good stability, as is shown by the low K_{11}' values for benzimidazole, purine, and guanine.

Theophylline anion is notably less effective as a complexing agent than is the un-ionized form. If hydrogen bonding made an important contribution to the stability of the complex, this behavior could be rationalized, but the results with caffeine do not support this view. (It is assumed that the gross structures of the xanthine complexes are the same.) An alternate hypothesis is that the anionic form is more effectively solvated, and the solvation shell decreases its capabilities for complexing with the substrate. A similar argument should apply to the 8-chlorotheophylline anion, though for this ligand the chlorine atom may aid interaction by a dispersion mechanism.

In theophylline-7-acetate the negative charge is localized rather than distributed into the rings. The resultant effect is not readily analyzed because for this system K_{11}' does not appear to be a simple 1:1 stability constant. This ligand can, however, be compared with caffeine, which it closely resembles except for a local negative charge and increased bulk in the 7 posi-

librium extent of complex formation, is to be understood, while by complex *reactivity* is meant the susceptibility of the complex species to reaction other than dissociation into its components.

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

(52) L. Pauling and R. B. Corey, *Arch. Biochem. Biophys.*, **65**, 164 (1956).

(53) (a) R. S. Schnaare and A. N. Martin, *J. Pharm. Sci.*, **54**, 1707 (1965); (b) B. Pullman and A. Pullman, *Proc. Natl. Acad. Sci. U. S.*, **44**, 1197 (1958); (c) R. D. Brown and M. L. Heffernan, *Australian J. Chem.*, **12**, 543 (1959).

tion. Either effect could be responsible for the decreased stability.

The guanine anion probably possesses a charge distribution greatly different from xanthine anions. This difference may be the cause of the much lower complexing tendency of guanine. Another possible contribution to complexing tendency is N-methylation, which is more extensive with the good ligands than with the less effective ones in the purine systems.

Types of Intermolecular Forces. Little can be said with assurance about the interaction forces responsible for the formation of these complexes. In the presence of some of the ligands the absorption band of the substrate showed a large change that might be described as either a bathochromic shift or an increase in intensity of absorption; because of the large background absorption by the substrate it was not possible to establish the absorption spectrum for a complex. Nevertheless, the considerable spectral change suggests that a charge-transfer type of interaction may be occurring. A very rough correlation may be made between the stability constants with these ligands and their electron donor abilities as predicted for the neutral molecules.[53b,54] Uric acid has been predicted to be a better donor than the xanthines,[53b] but its low solubility prevented its use.

Though hydrogen bonding between substrate and ligand may play a part in complexing by imidazole and 2-methylimidazole, it is not involved in the xanthine complexes, for with most of these systems neither the ligand nor the substrate possesses a hydrogen atom capable of forming a hydrogen bond. Hydrophobic bonding, the result of dispersion forces involving the ligand methyl groups, may be important, as may local dipole–dipole attractive forces. The contribution of solvent structure in stabilizing complex species could be a major factor. As mentioned earlier, increased solvation of theophylline anion may account for its decreased effectiveness relative to theophylline.

(54) A. Szent-Györgyi, "Introduction to a Submolecular Biology," Academic Press Inc., New York, N. Y., 1960.

Conclusions. The rate of alkaline hydrolysis of methyl *trans*-cinnamate is decreased in the presence of numerous imidazole and purine derivatives. This rate inhibition is attributed to the formation of relatively unreactive complexes between the ester and the heterocyclic compound. Though the stereochemistry of these complexes is unknown, it is probable that both steric and electronic effects are responsible for their diminished reactivity.

One consequence of possible importance to studies of homogeneous catalytic mechanisms is the effect of an unsuspected inhibition upon catalytic kinetic data. If an agent is capable of effecting both a catalysis and an inhibition, measurements of either phenomenon may be misinterpreted if the intervention of the other is not taken into account. On the basis of past work into mechanisms of ester reactions, it seems probable that the ligands employed here are not responsible for significant levels of catalytic activity against methyl cinnamate. It is quite possible, however, that in studies of the catalytic action of these or similar compounds a concurrent complexation may occur, and this will lead to spurious catalytic constants. Such behavior might result in nonlinear Brønsted plots (with negative deviations), for both the extent of complexation and the proportionate error it introduces will vary with the structure of the substrate and ligand.

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