Stability and Structure of Some Organic Molecular Complexes in Aqueous Solution

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Abstract □ On the basis of a simple model of 1:1 complex formation between planar molecules, this equation was derived: $\Delta G^0_u = A(G^0_{SL} - G^0_{MS} - G^0_{ML})$, where ΔG^0_u is the standard unitary free energy change for complex formation, A is the area of overlap in the plane-to-plane interaction between the two molecules, and G^0_{IJ} is a free energy of interaction per unit area between I and J. (S, L, and M represent substrate, ligand, and medium.) If the parenthesized term is roughly constant for a series of substrates and ligands in a constant medium, it is suggested that the quantity G^0_{SL} does not play a major role, and that the complex stability is primarily controlled by repulsive solvent interaction terms (G^0_{MS} and G^0_{ML}). It follows that the system will assume an orientation of S and L such as to maximize A. Therefore, maximal overlap areas were estimated for 18 neutral and 32 ionic complexes. A substantial improvement in the area correlation was obtained, and the observation was made that there is no significant difference between the average behavior of neutral and charged complexes. The maximal overlap concept provides a general "first-order" description of molecular complex formation in aqueous solution. Deviations from the line are significant and may be associated with specific structure-stability relationships. Application of the maximal overlap approach leads to average complex structures without introducing arbitrary assumptions about specific group interactions. The structures thus generated are consistent with the chemical reactivity of the complexes.

Keyphrases □ Complexes, organic molecular—aqueous solution □ Stability constants—organic molecular complexes □ Maximal overlap areas, estimated—complexes □ Titration, potentiometric, nonaqueous—analysis □ IR spectrophotometry—structure □ NMR spectroscopy—structure

The earlier suggestion (1) that systematic structural variations in substrates and ligands might be a successful approach to the elucidation of the structures of molecular complexes in solution formed the basis for much of the experimental design, including some of the present work. During the course of these structural investigations, it became increasingly apparent that the authors were studying secondary (specific) effects without having first identified the primary general effect in determining complex stability. This recognition led directly to the conception of the correlation between complex stability and planar area of interactants as described earlier (2). Complexes of neutral interactants gave a rough linear correlation of standard free energy change with planar area of the small interactant; this was interpreted in terms of a simple model of the process. Complexes containing an ionic interactant could not be usefully correlated, and it was suggested that neutral and ionic complexes behave differently in these systems.

The present work was planned to refine the area correlation and to elucidate the differences between neutral and ionic molecular complexes in aqueous solution. An ultimate goal is the ability to determine and to predict complex structures and properties (especially chemical reactivity) in solution, and the area correlation offers a promising point of attack.

EXPERIMENTAL

Materials—Many of the chemicals were prepared or purified as described elsewhere (1, 2). 3-β-Naphthylacrylic acid was prepared from β-naphthaldehyde[1] by the method of Fulton and Robinson (3), and its methyl ester was obtained by treatment with diazomethane. The slightly yellow product was recrystallized twice from ethanol–water, m.p. 88–89° [lit. (4) 93–93.5°]. IR and NMR spectra were consistent with the expected structure (5). Methyl 9-anthroate was prepared by treating 9-anthracene carboxylic acid chloride[1] with methanol in pyridine; it was recrystallized twice from ethanol–water, m.p. 110–111° [lit. (6) 111°]. 2,6-Naphthalenedicarboxylic acid dimethyl ester[2] was used directly, m.p. 185° [lit. (7) 186°]. Naphthalene[3] (resublimed) was used directly, m.p. 79° [lit. (8) 80.3°]. Phenanthrene[1] was recrystallized from ethanol, m.p. 99° [lit. (9) 101°]. The acid chloride of *p*-nitro-*trans*-cinnamic acid was prepared by refluxing the acid (Eastman White Label) with freshly distilled thionyl chloride. The acid chloride was refluxed with methanol to give the methyl ester, which was recrystallized twice from ethanol, m.p. 163° [lit. (10) 161°].

Benzimidazole[1] was recrystallized three times from water, m.p. 172–173° [lit. (11) 171–173°]. Benzimidazole 2-acetonitrile[1] was used directly, m.p. 209–210° [lit. (12) 209.7–210.7°]. 6-Nitrobenzimidazole[1] was recrystallized three times from methanol, m.p. 207–208° [lit. (13) 206°]. Xanthine and hypoxanthine[1] were used directly. Adenine[1] was recrystallized from water and dried at 150° for 8 hr. Nonaqueous titration in glacial acetic acid with acetous perchloric acid (quinaldine red indicator) showed that it was the anhydrous form. 8-Nitrotheophylline[2] was used directly, m.p. 282–283° dec. [lit. (14) 282–283° dec.]. Potentiometric titration with standard base indicated that it was the dihydrate. Theophylline[4] was recrystallized from water, dried at 150° for 4 hr., and then ground to a fine powder and dried for 24 hr., m.p. 270–271°. Nonaqueous titration in *N*,*N*-dimethylformamide with standard sodium methoxide in benzene–methanol solution (thymol blue indicator) showed it to be 100% anhydrous theophylline. Theobromine[4] was recrystallized from water and its purity checked by nonaqueous titration.

3,5-Dinitrobenzoic acid (Eastman White Label) was recrystallized twice from ethanol–water, m.p. 205–207° [lit. (15) 205–207°].

The pKa values were determined spectrophotometrically for many of the ligands; these values were found at 25° in water (ionic strength 0.3 M): 8-nitrotheophylline, 3.55 ± 0.05; hypoxanthine, 8.50 ± 0.06; xanthine, 9.95 ± 0.05 (for conversion to the anion); benzimidazole 2-acetonitrile, 4.20 ± 0.02 and 11.76 ± 0.02; and 6-nitrobenzimidazole, 2.89 ± 0.03 and 10.69 ± 0.05.

Apparatus and Procedures—The equipment and methods employed for stability constant determinations have been described in detail earlier (1, 2, 5, 16). Stability constants were evaluated by the solubility, spectral, and kinetic techniques (17) and are expressed as apparent 1:1 stability constants (K_{11}') in M^{-1}. All constants were determined at 25.0° in aqueous solution of 0.30 M ionic strength; in spectral and kinetic studies the solvent usually contained 0.83% acetonitrile, added as the substrate stock solution. An additional parameter provided by the kinetic method is symbolized q_{11} and is interpreted as the fractional decrease in chemical reactivity of the complexed substrate relative to the uncomplexed substrate (16).

RESULTS

Table I lists the substrate–ligand combinations studied, with their stability constants. Systems were investigated by the solubility

[1] Aldrich Chemical Co., Milwaukee, WI 53210
[2] K and K Laboratories.
[3] J. T. Baker, Phillipsburg, N. J.
[4] NF, Merck & Co., Inc., Rahway, NJ 07065

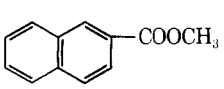
Table I—Apparent Stability Constants at 25° in Aqueous Solution

Substrate	Ligand	K_{11}' (M^{-1}) Solubility	Spectral	Kinetic
Methyl *trans*-cinnamate	Theophylline	25.0	24.5	—
Methyl *trans*-cinnamate	Theophyllinate[a]	—	13.2	13.5
Methyl *trans*-cinnamate	Theobrominate[b]	—	11.6	11.5
Methyl *trans*-cinnamate	8-Bromotheophyllinate	29	28[c]	—
Methyl *trans*-cinnamate	8-Iodotheophyllinate	33	—	—
Methyl *trans*-cinnamate	8-Nitrotheophyllinate	40[d]	—	—
Methyl *trans*-cinnamate	Benzimidazole	—	3.0[e]	—
Methyl *trans*-cinnamate	Benzimidazole cation	5.7	—	—
Methyl *trans*-cinnamate	Benzimidazole 2-acetonitrile cation	5.5	6.0	—
Methyl *trans*-cinnamate	Xanthine anion	—	7.5	—
Methyl *trans*-cinnamate	Hypoxanthine anion	—	2.0	—
Methyl *trans*-cinnamate	Adenine cation	—	2.5	—
Methyl *trans*-cinnamate	3,5-Dinitrobenzoate[f]	9.4	—	—
Methyl 1-naphthoate	Theophyllinate	—	—	25
Methyl 1-naphthoate	Theobrominate	—	—	36
Methyl 1-naphthoate	8-Chlorotheophyllinate	—	—	64
Methyl 2-naphthoate	Theophyllinate	—	—	50
Methyl 2-naphthoate	Theobrominate	—	—	39
Methyl 2-naphthoate	8-Chlorotheophyllinate	120	—	105
Methyl 2-naphthoate	8-Nitrotheophyllinate	230	—	—
Methyl 3-β-naphthylacrylate	Theophyllinate	—	—	41
Methyl 3-β-naphthylacrylate	Theobrominate	—	—	30
Methyl 3-β-naphthylacrylate	8-Chlorotheophyllinate	—	—	58
Methyl *p*-nitrocinnamate	Theophyllinate	—	—	13.5
Methyl 9-anthroate	8-Chlorotheophyllinate	85	—	—
Naphthalene 2,6-dicarboxylic acid, dimethyl ester	8-Chlorotheophyllinate	160	—	—
Cinnamamide	Theophylline	25	—	—
trans-Cinnamic acid anion	Theobrominate	—	5	—
Naphthalene	Theophylline	64	—	—
Naphthalene	Theophyllinate	23	—	
Naphthalene	8-Chlorotheophyllinate	49	—	—
Phenanthrene	Theophyllinate	89	—	—
3,5-Dinitrobenzoate	Theophylline	—	10.3	—
3,5-Dinitrobenzoate	Theophyllinate	—	7.5	—
3,5-Dinitrobenzoate	8-Chlorotheophyllinate	—	13.4	—

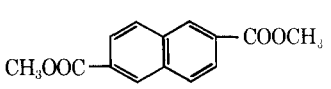
[a] *I.e.*, theophylline anion. [b] Theobromine anion. [c] B. J. Kline, Ph.D. dissertation, Univ. of Wisconsin, Madison, Wis., 1968. [d] 1:1 stoichiometry observed [e] Same value found at two wavelengths. [f] 3,5-Dinitrobenzoic acid anion.

method (*18*) when substrate stability and ligand solubility permitted. Spectral determinations were made on those systems in which a measurable spectral shift occurred upon complexation. The kinetic technique was applied to most of the ester substrates. The reproducibility of most stability constants is about ±10% (the cinnamic acid anion–theobrominate constant is only reliable to ±25% because it was estimated in "infinity" solutions from a kinetic study of methyl cinnamate–theobrominate).

Nearly all of the systems studied include one heterocyclic interactant (methyl cinnamate–3,5-dinitrobenzoate is the only exception), so this limitation must be recognized. As noted earlier, most of the substrates and ligands were selected to provide information on the specific structural requirements for complexing and on the nature of ionic complexes. Another possible feature that was considered was the statistical effect on apparent complex stability (*18*). Thus, methyl 2-naphthoate (I) and 8-chlorotheophyllinate form a complex with $K_{11}' = 120\ M^{-1}$. If 8-chlorotheophyllinate interacts in a specific orientation with methyl 2-naphthoate, and if in a 1:1 complex of 8-chlorotheophyllinate with the dimethyl ester of naphthalene 2,6-dicarboxylic acid (II) this orientation is preserved, then K_{11}' for the complex with II should be twice 120. The observed value is 160 M^{-1}, possibly indicating either that there

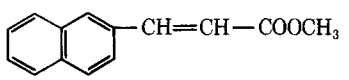


I



II

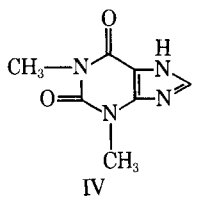
is no specific orientation of the ligand common with I and with II, or that the binding energy of the ligand with II is influenced by the second ester group. Again, comparing the complex naphthalene–theophyllinate ($K_{11}' = 23\ M^{-1}$) with phenanthrene–theophyllinate ($K_{11}' = 89\ M^{-1}$) shows that a simple statistical effect is not the only factor controlling the constant with the larger substrate. (Anthracene could not be studied because of its very low solubility.)



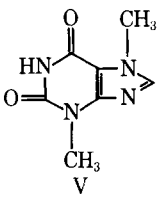
III

Methyl 3-β-naphthylacrylate (III) can be viewed either as a 2-naphthoate with a conjugated side chain or as a methyl cinnamate derivative with a *m,p*-fused aromatic ring. A ligand has a "choice" of positions, one of them characteristic of a naphthoate substrate and one resembling a cinnamate. Comparison of the stability constants for III, for methyl cinnamate, and for methyl 2-naphthoate with common ligands suggests that the ligand preferentially resides at the naphthyl portion of III. This conclusion is strengthened by the q_{11} values, which will be considered later Also consistent with this interpretation is the absence of a useful spectral shift upon complexation, which is typical of benzoate and naphthoate complexes but not of cinnamate complexes.

Some of these data suggest that charge-transfer and simple electrostatic forces are not of major importance in determining complex stability in aqueous solution. The anions of theophylline (IV) and theobromine (V) have very different electronic distributions, but they yield similar K_{11}''s with the common substrate methyl *trans*-cinnamate.



IV



V

Cinnamic acid anion complexes appreciably with the anions theophyllinate (4) and theobrominate. Methyl *p*-nitrocinnamate

Table II—q_{11} Values for Alkaline Hydrolysis of Ester Complexes[a]

Substrate	Ligand	q_{11}
Methyl *trans*-cinnamate	Theophyllinate	0.90
Methyl *trans*-cinnamate	Theobrominate	1.0
Methyl 1-naphthoate	Theophyllinate	0.97
Methyl 1-naphthoate	Theobrominate	0.90
Methyl 1-naphthoate	8-Chlorotheophyllinate	1.0
Methyl 2-naphthoate	Theophyllinate	0.91
Methyl 2-naphthoate	Theobrominate	0.93
Methyl 2-naphthoate	8-Chlorotheophyllinate	0.99
Methyl 3-β-naphthylacrylate	Theophyllinate	0.71
Methyl 3-β-naphthylacrylate	Theobrominate	0.82
Methyl 3-β-naphthylacrylate	8-Chlorotheophyllinate	0.89
Methyl *p*-nitrocinnamate	Theophyllinate	0.82

[a] 25°, 0.3 *M* ionic strength, phosphate or hydroxide buffers, 0.83% acetonitrile.

interacts with theophyllinate to about the same extent as does methyl cinnamate. The anion of 3,5-dinitrobenzoic acid, which Menger and Bender (19) expected to function as an electron acceptor, complexes significantly with several anions (Table I). Thus, with theophylline, a K_{11}' of 10 M^{-1} is observed. Yet a stability constant of 9 M^{-1} is found for the 3,5-dinitrobenzoate–methyl cinnamate complex, and methyl cinnamate complexes with theophylline with K_{11}' equal to 25 M^{-1}. Simple donor–acceptor relationships do not provide a convincing picture of this cycle of numbers.

3,5-Dinitrobenzoate gave $K_{11}' = 13\ M^{-1}$ with 8-chlorotheophyllinate, whereas Guttman and Brooke (20) found a stability constant of 4.7 M^{-1} between 8-chlorotheophyllinate and 3-carbomethoxy-1-methylpyridinium cation; that is, the 8-chlorotheophylline anion interacts more strongly with another anion that it does with a cation.

Earlier results (16) led to an estimate of the uncertainty in q_{11} of about 0.1 unit (q_{11} is constrained, for 1:1 complexes exhibiting inhibition relative to the uncomplexed substrate, to lie in the interval 0–1). In the present study the reactions were simple ester hydrolyses in easily analyzed and controlled systems, and it is estimated that most of the q_{11} values have an uncertainty of about 0.05 unit. These q_{11} values are collected in Table II. Figure 1 shows a plot illustrating the determination of these quantities (16, 17); q_{11} is equal to the reciprocal of the intercept on the ordinate axis.

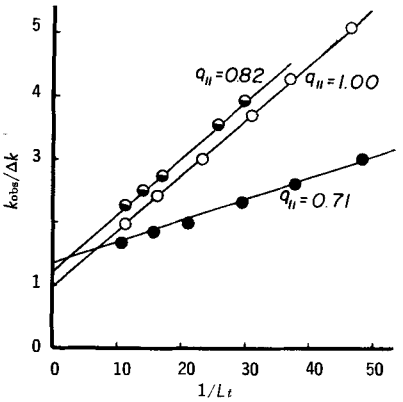


Figure 1—*Kinetic plot showing several complex systems* (L_t *is total ligand concentration*). *Key:* ○, *methyl* trans-*cinnamate–theobrominate;* ◒, *methyl* p-*nitrocinnamate–theophyllinate; and* ●, *methyl 3-β-naphthylacrylate–theophyllinate.*

DISCUSSION

Maximal Overlap Area Correlation—The model of complex formation presented earlier (2) assumes plane-to-plane interaction of substrate *S* and ligand *L* in medium *M*. Equation 1 can be developed from this model:

$$\Delta G_u^0 = A(G_{SL}^0 - G_{MS}^0 - G_{ML}^0) \qquad \text{(Eq. 1)}$$

where ΔG_u^0 is the standard unitary free energy change for complex formation (21), G_{IJ}^0 is a free energy of interaction per unit area between "surfaces" *I* and *J* at the equilibrium separation distance, and *A* is the area of contact between *S* and *L*. The first use of this model took *A* to be the estimated planar area of the smaller of the two interactants (2). A plot of standard unitary free energy change against planar area of the smaller interactant gave a fair linear correlation for complexes in which both *S* and *L* were neutral, but the attempted correlation was not successful when either, or both, of the interactants was an anion. An obvious fault of the treatment is that numerous points corresponded to the same area (because they involved one of the same interactants) yet represented different complex stabilities. This generated vertical lines in the plot. If the model is a good one for complex formation, part of the lack of success apparently was caused by a poor estimate of the overlap area *A*. *A* cannot be greater than the planar area of the smaller interactant, but it may be smaller than this quantity. A refined estimate of the overlap area was made as follows.

A free energy term of the type G_{IJ} assumes an increasingly negative value as attraction between *I* and *J* increases. (When *I* and *J* are separated to infinity, G_{IJ} equals zero.) In a first approximation, the surfaces are considered homogeneous; that is, no preferred specific (group) interactions are considered. Then *A* will assume a value such as to maximize $-\Delta G^0$ subject to these conditions: (*a*) the more negative the value of G_{SL}^0, the greater *A* will be; (*b*) the more positive the value of G_{MS}^0, the greater *A* will be; and (*c*) the more positive the value of G_{ML}^0, the greater *A* will be. In chemical terms, attraction between *S* and *L* will lead to a maximization of overlap area, whereas repulsion would tend to minimize the area. Attraction between *M* and *S* will minimize overlap area, whereas *M–S* repulsion will maximize it, and similarly for *M* and *L*. The final structure is then a compromise position depending upon (possibly) opposing factors.

Since most of these compounds are relatively hydrophobic, it is reasonable to suppose that G_{MS}^0 and G_{ML}^0 are fairly large repulsive quantities. That G_{SL}^0 does not play an overwhelmingly important role is indicated by the marked reduction in K_{11} for the methyl cinnamate–theophylline complex as the water concentration is decreased in water–methanol mixtures (22). It seems possible, therefore, that in many of these complexes a structure is assumed in which the overlap area between substrate and ligand is maximized. Therefore, a plot was made of standard unitary free energy change, ΔG_u^0, against *maximal overlap area.*

Maximal overlap areas were estimated as shown by Fig. 2. The planar area of the smaller molecule is traced from a Corey-Pauling-Koltun molecular model (23) on translucent tracing paper and is darkened. The planar area of the larger molecule is traced on a transparent plastic sheet. These are superimposed and oriented until the area of overlap is visually maximized, with nothing else being taken into consideration. A photocopy of this orientation is made, and the overlap area (hatched area in Fig. 2) is measured by a planimeter. This gives the maximal overlap area for plane-to-plane interaction between the two molecules. Table III gives maximal overlap areas estimated in this way.

Figure 3 shows the correlation of ΔG_u^0 with maximal overlap area for neutral complexes (dark circles). The correlation seems to be slightly better than the original plot against the planar area of the smaller interactant (2). The open circles in Fig. 3 represent complexes in which one or both interactants are ionic. A marked improvement in the correlation of ionic complexes has been achieved with the maximal overlap area concept.

The 50 complexes plotted in Fig. 3 represent about 70 systems, because, for clarity, one point may represent more than one complex. For example, the methyl *trans*-cinnamate–theophylline complex has the same stability constant and overlap area as do several other cinnamate–theophylline complexes, so these additional points were not plotted. A least-squares line calculated for the 50 points gave an ordinate intercept of 0.1 (in units of $-10^{21}\ \Delta G_u^0/N$), indicating that the line passes essentially through the origin, in agreement with the model equation. Separate least-squares lines for the neutral and ionic complexes gave the same slope values, with the standard deviation for neutral complexes being 0.58 and for ionic complexes 0.64, in units of $-\ 10^{21}\ \Delta G_u^0/N$. This treatment has, there-

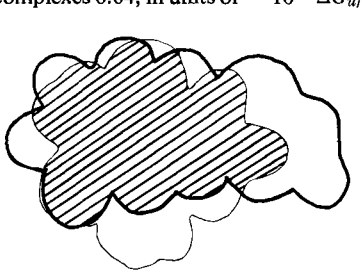


Figure 2—*Maximal overlap area (hatched area) in the methyl* trans-*cinnamate–theophylline complex. Methyl cinnamate is outlined with the heavier line, with the ester group lying to the right.*

Table III—Estimated Maximal Overlap Areas for Complexes

System Number	Substrate	Ligand	Maximal Overlap Area (A^2/molecule)
1	Methyl 2,6-dichloro *trans*-cinnamate	Theophylline	55.6
2	Acetophenone	Theophylline	44.4
3	Methyl *trans*-cinnamate	Benzimidazole	39.5
4	Naphthalene	Benzimidazole	41.2
5	Methyl 1-naphthoate	Theophylline	54.7
6	*trans*-Cinnamaldehyde	Theophylline	49.4
7	Benzalacetone	Theophylline	49.8
8	Methyl *trans*-cinnamate	2-Methyl imidazole	28.4
9	Benzamide	Theophylline	39.5
10	Naphthalene	Theophylline	49.8
11	Methyl crotonate	Theophylline	27.2
12	Methyl benzoate	Theophylline	39.9
13	Methyl hydrocinnamate	Theophylline	34.2
14	*trans*-Cinnamyl acetate	Theophylline	37.9
15	Methyl 2-naphthoate	Theophylline	53.1
16	Methyl *trans*-cinnamate	Theophylline	49.0
17	Methyl *trans*-cinnamate	Caffeine	46.9
18	*trans*-Cinnamamide	Theophylline	45.3
19	Methyl *trans*-cinnamate	Purine anion	33.3
20	Theophylline	Salicylate	39.1
21	Methyl *trans*-cinnamate	Hypoxanthine anion	35.4
22	Methyl *trans*-cinnamate	8-Bromotheophyllinate	48.1
23	Methyl *trans*-cinnamate	3,5-Dinitrobenzoate	39.9
24	Cinnamoylsalicylate	Theophylline	44.9
25	Theophylline	*trans*-Cinnamate	42.4
26	Theophylline	3,5-Dinitrobenzoate	51.9
27	Methyl *cis*-cinnamate	8-Nitrotheophyllinate	44.0
28	Methyl *trans*-cinnamate	8-Nitrotheophyllinate	53.9
29	Methyl 2-naphthoate	8-Nitrotheophyllinate	55.6
30	Methyl *trans*-cinnamate	8-Chlorotheophyllinate	47.8
31	Methyl 2-naphthoate	8-Chlorotheophyllinate	55.6
32	Methyl 1-naphthoate	8-Chlorotheophyllinate	51.0
33	Naphthalene	8-Chlorotheophyllinate	46.5
34	Phenanthrene	8-Chlorotheophyllinate	56.0
35	9-Methyl anthroate	8-Chlorotheophyllinate	56.8
36	Naphthalene 2,6-dicarboxylic acid, dimethyl ester	8-Chlorotheophyllinate	54.3
37	Methyl 3-β-naphthylacrylate	8-Chlorotheophyllinate	54.3
38	Methyl crotonate	Theophyllinate	27.2
39	*p*-Nitrophenyl benzoate	Theophylline	38.7
40	Methyl *cis*-cinnamate	Theophyllinate	42.0
41	Methyl 2-naphthoate	Theophyllinate	53.9
42	Methyl 1-naphthoate	Theophylinate	53.5
43	Methyl *trans*-cinnamate	Theophyllinate	50.2
44	Methyl 3-β-naphthylacrylate	Theophyllinate	51.4
45	Methyl *p*-nitrocinnamate	Theophyllinate	50.2
46	Naphthalene	Theophyllinate	49.0
47	Methyl *trans*-cinnamate	Theobrominate	47.3
48	Methyl 3-β-naphthylacrylate	Theobrominate	49.8
49	Methyl 1-naphthoate	Theobrominate	51.0
50	Methyl 2-naphthoate	Theobrominate	51.0

fore, revealed the result that, in the first-order approximation, all systems behave similarly and, *on the average*, there is no difference between neutral and ionic complexes. This was not obvious on the basis of data for systematically selected substrates and ligands, and it only became clear when so many systems had been studied that the selection was practically random.

The equation of the line in Fig. 3 is

$$-\Delta G_u^0/N = 0.149 \times 10^{-21}\ (\text{cal.}/A^2) \times \text{maximal overlap area } (A^2/\text{molecule}) \quad \text{(Eq. 2)}$$

This slope value is equivalent to 64 dyne/cm. If, as suggested, the *S–L* interaction is of minor importance in determining complex stability, then the average value of the G_{MS}^0 and G_{ML}^0 terms is 32 dyne/cm. This is approximately the value of interfacial tensions between water and some typical hydrophobic organic compounds (24).

The statistical nature of this first-order approximation is apparent from Fig. 3. If another large group of structurally similar substrates and ligands was studied, the points would be expected to define a line close to the one in the figure. However, no single stability constant value could be predicted to within better than a factor of two. The dispersion seen in Fig. 3 may be considered a second-order effect, probably correlatable with specific structural features in the substrate and ligand. (Most of the deviations from the line are too large to be ascribed to misestimates of maximal overlap areas.) In terms of the model and Eq. 1, the quantity ($G_{SL}^0 - G_{MS}^0 - G_{ML}^0$) varies appreciably within the series of complexes represented. This scatter of points does not invalidate the model, which does not include the constraint that the parenthesized quantity should remain constant as *S* and *L* are changed.

Few general inferences can be made at this time concerning the second-order effect (the deviations from the area correlation line) in describing complex stability. One interesting set of data is the stability constants for theophylline and theophyllinate complexes with seven common substrates; the constant for the neutral ligand is nearly always larger than that with the anion, the mean value of the ratio being 2.35 ± 0.73. This is explicable if it is considered that the anion is less hydrophobic than is the neutral ligand, resulting in a more negative G_{ML}^0 term and, hence, decreased complex stability. (Since theophylline was the only ligand that could easily be studied in its neutral and anionic forms, these data earlier led to the too general belief that ionic complexes always behave differently from neutral ones.) That this solvation effect is not the only factor is indicated by stability constants for six common substrates with theophyllinate and 8-chlorotheophyllinate; the ratio of these constants (8-chlorotheophyllinate–theophyllinate) is 2.01 ± 0.33. The larger area of the halogenated xanthine does not account for the effect. These examples indicate a complicated variation in the energy terms even for closely related compounds.

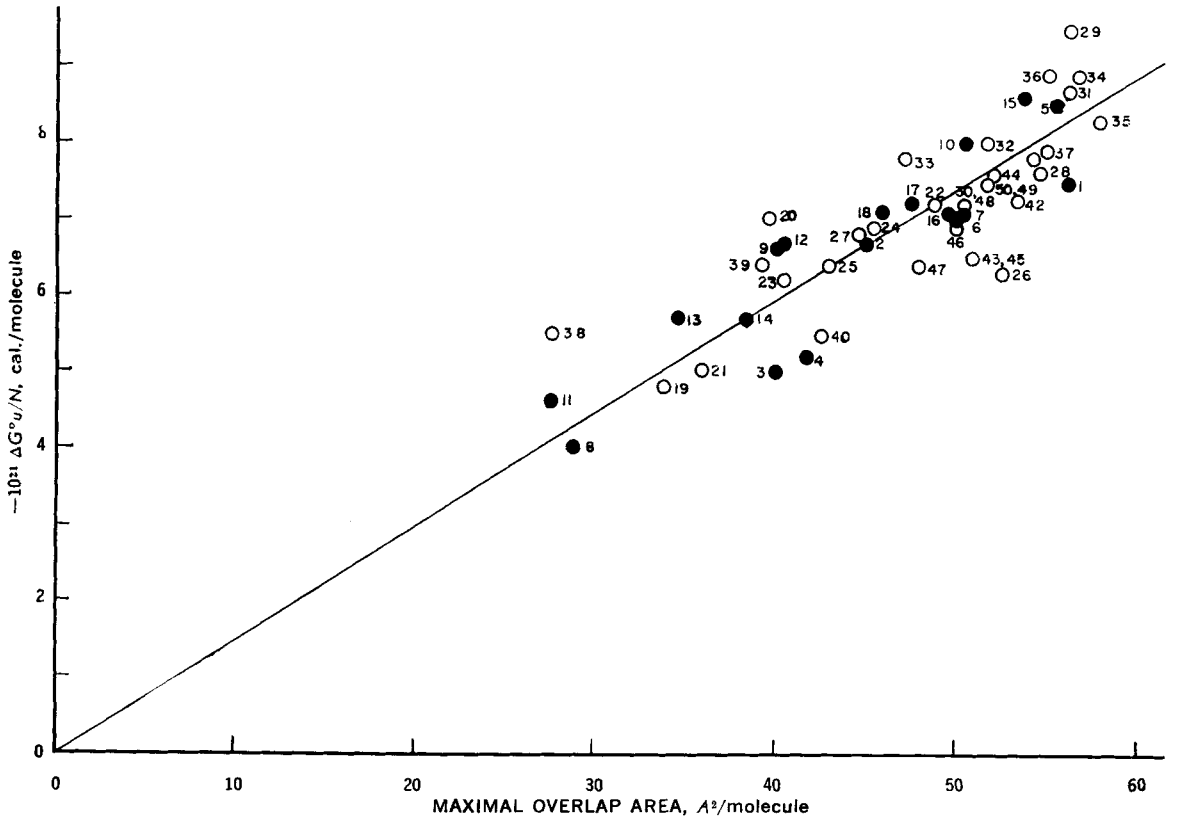


Figure 3—*Plot of standard unitary free energy change for complex formation at 25° in aqueous solution against estimated maximal overlap area. Key: ○, ionic complexes; and ●, neutral complexes. See Table III for complex identities.*

Structure and Reactivity of Complexes—Previous reports have stated q_{11} values for ester hydrolysis to be either about unity (implying direct contact of the ligand with the ester group and, therefore, substantial inhibition of the reaction) (1, 2, 16) or less than 0.5 (with no direct steric interference but a small polar effect on the rate). In this study, ester substrates have been selected to allow a distinction to be made between q_{11} values in the range 0.7–1 (Table II and Fig. 1).

Repetition of the methyl *trans*-cinnamate–theophyllinate study (1) yielded q_{11} = 0.90, which is now thought to be significantly different from unity. The methyl *trans*-cinnamate–theobrominate complex, however, gave a q_{11} value of essentially unity. The stability constants and maximal overlap areas for the two complexes are about the same. The maximal overlap area profiles, developed as described earlier, suggest a possible reversal of the xanthine–cinnamate orientation, with the xanthine occupying a position closer to the ester carbonyl when the ligand is theobrominate; this is consistent with the higher q_{11} value.

Methyl 3-β-naphthylacrylate (III) is a larger molecule than methyl *trans*-cinnamate, and it seemed that a kinetic study with theophyllinate might define the area of interaction between III and this ligand. The q_{11} of 0.71 implies only a small steric involvement with the ester function (a q_{11} of 0.71 corresponds to 0.73 kcal./mole difference in free energy of activation between the complexed and uncomplexed substrate), suggesting that the xanthine lies further away from the ester group than it does in the methyl cinnamate complex. Further, the q_{11} of 0.89 for the 8-chlorotheophyllinate complex of III is consistent with the greater bulk of this xanthine and the possibility that greater steric interference occurs by the ligand in the reaction of the ester function.

It is curious, considering the scatter observed in the area correlation (Fig. 3), that the maximal overlap concept should consistently lead to a picture of the substrate–ligand orientation (that is, the complex structure) that is reasonable in terms of measurable complex reactivity. This leads to the tentative view that the maximal overlap area approach provides a realistic estimate of complex structures in aqueous solution, including an estimate of average complex stability. The actual stability of a particular complex is determined, within this average configuration, by local atomic and group interactions.

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Central Hypotensive Activity of *dl*- and *d*-Propranolol

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Abstract □ The intraventricular administration of *dl*-propranolol to α-chloralose-anesthetized cats was followed by a decrease in blood pressure and was devoid of an associated tachycardia. *d*-Propranolol in an equivalent dose produced a hypotensive response which was not statistically different from *dl*-propranolol. These data suggest that there is a central component in the hypotensive response to propranolol and that it is independent of the β-adrenergic blocking activity. The intraventricular administration of 500 mcg. of reserpine base produced greater than a 90% depletion of norepinephrine in all brain regions analyzed within 24 hr. This pretreatment with reserpine also reversed the hypotensive response to both *d*- and *dl*-propranolol as well as converted the decrease in heart rate to an increase in heart rate. Thus, at least one of the amines must be required to produce the hypotensive response. Perfusion of the ventricular system with *dl*-propranolol generally produced an increase in epinephrine along with a decrease in norepinephrine in the brain region analyzed. Under the same conditions, *d*-propranolol also increased epinephrine but led to a decrease in norepinephrine in only 50% of the tissues assayed. These changes in amine levels occurred during the perfusion, which also produced a sustained hypotensive effect lasting the duration of the perfusion. The hypotension associated with propranolol therapy may have a central component that is not dependent on the β-adrenergic blocking property of propranolol, which requires one or more of the brain amines, and leads to an increase in the epinephrine level along with a general decrease in the norepinephrine level of the brain.

Keyphrases □ *dl*-, *d*-Propranolol—central hypotensive activity □ Hypotensive action, *dl*-, *d*-propranolol—intraventricular injection □ β-Adrenergic blocking activity—propranolol □ Catecholamines, brain—propranolol effect

Propranolol antagonizes the cardiovascular effects of β-adrenergic receptor stimulation produced by either stimulation of effector fibers or by sympathomimetic amines. The intravenous administration of propranolol produces a decrease in the sympathetic component to the heart and blocks the chronotropic effects of isoproterenol and epinephrine and the peripheral vasodilatory effects of isoproterenol (1–3). The acute response to propranolol is a decrease in cardiac output, whereas prolonged administration fails to produce this effect (4). Several reports have shown that the intravenous administration of propranolol to normotensive or hypertensive individuals decreased heart rate and cardiac output along with a concomitant decrease in systemic blood pressure but exhibited no significant effects on systemic peripheral resistance (4–6). Epstein *et al.* (5) and Shinebourne *et al.* (6) reported that the increase in arterial pressure that is associated with exercise was abolished by propranolol *via* reduction in cardiac output.

The central nervous system (CNS) manifestations attributed to propranolol classify it as a sedative and general CNS depressant (7–9). Since both propranolol and pronethalol possess these properties while dichloroisoproterenol (DCI) produces CNS stimulation, these authors have concluded that it is the presence of the naphthyl group rather than the β-adrenergic blockade that is responsible for the CNS effects. The depression or tranquilization in humans associated with propranolol administration is invariably apparent only at dosage levels that are many times greater than those necessary to produce β-adrenergic blockade (10–12). Masuoka and Hansson showed that intravenous administration of ^{14}C-labeled propranolol is rapidly taken up by the rat brain and concentrated 50 times greater in the brain than in the blood (13).

The hypotensive effect induced by the administration of β-adrenergic blocking agents in man has been well documented (14–22). This response is not exclusive to humans, because other investigators also have noted depressor effects in animals (23–26). The oral administration of propranolol for several weeks has been reported to result in a gradual and significant decrease in systemic arterial pressure in hypertensive patients (10, 16, 22). In all cases, regardless of the time of onset, the dose of the β-adrenergic blocker was many times that required for the blockade of the β-receptors, thus leading to the hypothesis that this response may not be due to antagonism of these receptors. This hypotensive response was unexpected, since the blocking of a neural mechanism that produces vasodilation in the absence of a significant reduction in cardiac output might be expected to result in a rise in systemic blood pressure