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## RESEARCH ARTICLES

# Solvency and Hydrogen Bonding Interactions in Nonaqueous Systems

T. HIGUCHI, J. H. RICHARDS, S. S. DAVIS, A. KAMADA, J. P. HOU, M. NAKANO, N. I. NAKANO, and I. H. PITMAN

**Abstract** □ A study of the importance of hydrogen-bonding interactions in the formation of complexes is useful in providing knowledge of the physical and chemical properties of drug molecules and hopefully it will eventually lead to rational formulation of drugs into suitable dosage forms. The present report deals with a study of some of the methods available for the experimental measurement of these interactions and the means of determining the association (equilibrium) constants from the experimental results. An attempt is made to develop suitable methods for the quantitative analysis of hydrogen-bonding data so that useful estimates of association constants can be made *a priori*. The effect of the nonaqueous solvent on the value of the association constant is also discussed and a method is given whereby an estimate of solvent interaction can be calculated.

**Keyphrases** □ Solvency-hydrogen bonding interactions—nonaqueous systems □ Complex formation—hydrogen bonding interaction □ Solubility method—association constants □ Liquid-liquid partition method—association constants □ Optical rotatory dispersion method—association constants □ UV spectrophotometry method—association constants □ Linear free energy relationship—hydrogen bonding □ Solvent effects—stability constants, complexes

An attempt has been made in this paper to organize and bring together much of the current information available on the overwhelming role of hydrogen-bond formation on the properties of pharmaceutical and related solutions. A substantial portion of the material presented has been drawn from the literature, the remainder has been based on various published and unpublished studies carried out in the authors' laboratories at The University of Wisconsin and The University of Kansas. The present treatment has been limited

to the interactions of organic species in essentially nonaqueous systems.

#### DISCUSSION

Hydrogen-bond formation plays an extremely important role in controlling various physical processes of prime interest to the pharmaceutical chemist. Solubility, rate of dissolution, rate of zone migration in GLC, TLC, and paper chromatography, partition coefficient, rate of drug release, differential volatility, activity coefficients, *etc.*, are usually controlled by and predictable on the basis of hydrogen-bond formation.

Although, for example, various theories and hypotheses have been proposed in the area of solubility behavior of nonelectrolytes the most pharmaceutically useful approach appears to be that based on the concept that such solutions represent summation of effects arising from interactions of a large number of equilibrium systems. In nearly every instance the more important interactions in these solutions are due to rapid formation and breaking of hydrogen bonds. Thus, if we were to consider solvency of substance *A* in solvent *B* it is evident that

$$\text{total } A \text{ in solution} = [A] + 2[A_2] + 3[A_3] + \dots + [AB] + 2[A_2B] + 3[A_3B] + \dots + [AB_2] + 2[A_2B_2] + 3[A_3B_2] + \dots + \dots$$

where the various terms in the right part of the equation represent the various species present in solutions which contain one or more molecules of *A* per unit. The concentration of each species can be related to the monomer concentrations of *A* and *B* if the stability constant for the particular species was known. Thus, for example,

$$\begin{aligned} [A] &= K_1 : 0 \\ \text{and} & [AB] = K_{1:1} [A][B] \\ \text{and} & [AB_2] = K_{1:2} [A][B]^2 \\ \text{and} & [AB_n] = K_{m:n} [A]^m [B]^n \end{aligned}$$

It must, however, be kept in mind that  $[B]$  is not always equal to the reciprocal of the molar volume of *B* even for the pure solvent,

the monomeric concentration of *B* in many cases such as for alcohols, phenols, carboxylic acids, amides, glycols, amines, *etc.*, being markedly reduced by formation of dimers, trimers, *etc.*, arising from hydrogen bonding. It is evident, however, that it would be theoretically possible to calculate the overall solubility if all of the constants were available.

The present report is largely concerned for this reason with methods of experimentally determining these constants and with approaches by which they can be predicted on an *a priori* basis. The methods described include solubility, extraction, and various spectroscopic procedures which have yielded consistent results. A new correlative analysis is given which appears to be potentially highly useful in estimating these constants from limited amounts of data.

The concept of the hydrogen bond was first proposed by Latimer and Rodebush in 1920 (1) to explain the anomalous behavior of liquids such as water, hydrogen fluoride, and acetic acid. In recent years the hydrogen-bond concept has been used widely to explain molecular associations and interactions. Improved instrumentation and techniques such as IR, NMR, UV, and fluorescence spectroscopies, and X-ray diffraction have been especially useful in this field, not only in determining the existence of hydrogen bonds but also in providing information as to the nature of the bonds themselves (2-5).

Pimentel (2) has defined the hydrogen bond as that existing between a functional group X—H and an atom or a group of atoms of Y in the same or different molecules when (*a*) there is evidence of bond formation (association or chelation) and (*b*) there is evidence that this association involves bonding to a hydrogen atom already bonded to some other atom. The usual explanation of hydrogen bonding is that it is due primarily to an electrostatic interaction and calculations on this basis have given reasonable agreement with experimentally obtained values (6, 7). The hydrogen atom is considered to be unique in forming such electrostatic bonds because of its small size and the absence of inner shells of electrons. Nagakura and Gouterman (8) have suggested that in hydrogen donor-acceptor complex formation the hydrogen donor and acceptor act as electron acceptor and donor, respectively. This is analogous to Mulliken's (9, 10) charge transfer theory of molecular complex formation.

Several recent studies (11-15) have strongly emphasized that in addition to the essential electrostatic interaction, a charge transfer mechanism (delocalization effect) also plays an important role. Modern quantum mechanical treatment suggests that delocalization effects, repulsive forces, and dispersion forces as well as electrostatic forces contribute to the hydrogen bond (4, 5). Since a number of reviews on the theory of hydrogen bonding are available a detailed discussion will not be given here (2, 16, 17).

The hydrogen bond, X—H . . . Y is usually considered to be a weak chemical bond in which a hydrogen atom lies between the two closely spaced electronegative atoms, X and Y. The atoms X and Y are usually oxygen, nitrogen, fluorine, or chlorine atoms. Halogen-activated C—H, acetylinic C—H, and the S—H groups, however, can also act as hydrogen donors (2). There are also numerous data on the association of donor molecules with the  $\pi$  electrons of aromatic compounds (2).

Hydrogen bonds can be formed within molecules (intramolecular) as well as between molecules (intermolecular). The properties of a substance depend strongly on whether the molecule exists as cyclic, chelated individuals or is linked as straight or twisted chain polymers. For example, pure compounds that form intermolecular hydrogen bonds have high boiling points, high melting points, and high heats of vaporization. These effects are reduced or even lost if the hydrogen-bonding groups are placed so as to permit intramolecular bondings.

The most striking property of hydrogen-bonded complexes is the rapid reversibility of the association under normal conditions. The physical properties and some chemical properties are often markedly different from those of the parent donor or acceptor molecules. Accordingly, the equilibrium (stability) constant of the complex can be determined by a suitable technique.

Although the molecular associations resulting from hydrogen-bond formation are often regarded as being a well-defined 1:1 stoichiometry, recent investigations have revealed that only a few systems can be treated entirely in this simple fashion. Even chloroform, which contains only one functional polar hydrogen atom, has been shown to self-associate (18-20). Phenolic compounds in

**Table I—The Interaction of Phenols (and Phenolic Compounds) with Various Hydrogen Acceptors**

Hydrogen Acceptor	References
Ketones	25-28, 31
Ethers	25, 29-31
Aromatics	31-33, 42
Organophosphorus compounds	34-36
Alcohols	37
Vinyl compounds	34, 38
Amides	39
Esters	40
Griseofulvin	41

particular are known to exist in more than one hydrogen-bonded species in solution. At low concentrations the main equilibrium is between monomers and dimers (21, 22) although there is evidence that a cyclic structure can exist (23, 24). Such effects can have considerable implications on the computation of stability constants of the complex formed between phenol and bases. It is usually assumed that a cyclic dimer does not take part in association with the base, whereas the open dimer is a more effective donor than the monomeric species. At low concentrations it can be assumed that for phenol only the monomeric form exists. However, experimentally such low concentrations are only feasible in UV spectroscopy.

From a pharmaceutical standpoint the interaction of phenolic compounds with a great variety of hydrogen acceptors has provided much information as to the nature of complex formation. Table I lists some of the complexes that have been studied. The list is not intended to be exhaustive.

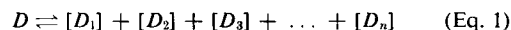
Recently Sellier and Wojtkowiak (43) and Purcell and Drago (44) have analyzed phenol-complex data and have calculated energies of hydrogen-bond formation from enthalpy values.

In view of the importance of hydrogen-bonding interactions in the formation of complexes, the present article will mainly be concerned with a study of the methods available for the experimental measurement of these interactions. An attempt will also be made to develop a method for the quantitative analysis of hydrogen-bonding data, so that useful estimates of association constants can be made *a priori*.

**Experimental Methods for the Determination of Association Constants for Hydrogen-Bonding Interactions in Nonaqueous Solution**—Four different methods are considered. Particular attention is paid to the methods by which association constants can be calculated from the experimental data.

*A. Solubility Method*—A detailed discussion of this method has been given by Kostenbauder (45). The solution is maintained saturated with respect to one component (*D*), and incremental amounts of complexing agent, proton acceptor (*A*) are added. At equilibrium the total amount of *D* in solution is determined spectrophotometrically. If the complexes formed are not highly soluble a plot of solubility against the amount of complexing agent added will increase proportionally until it reaches the saturation solubility of the complex itself, and then the curve will level off. If a very soluble complex is formed the curve will never reach a plateau but the stoichiometric formula of the complex can be investigated.

When there are only two components involved in the system in which solvent is saturated with proton donor *D*, the solute may exist as different associated species,



where the subscript refers to the several discrete states of aggregation of the solute in the solution. The chemical potential of each is

$$\sum_{n=1}^{\infty} nD_n = \text{observed molar solubility of } D \text{ in solvent } S = D_s$$

If into this system we introduce a third component, the second solute *A*, in a subsaturation amount capable of undergoing specific interaction with the first, and if we assume both compounds to be present in a relatively low concentration we can write the solute species as  $[D] + [D_2] + [D_3] + \dots + [D_n] + [A] + [A_2] + [A_3] + \dots + [A_n] + [DA] + [DA_2] + [DA_3] + \dots + [DA_n] + [D_2A] + [D_2A_2] + [D_2A_3] + \dots + [D_2A_n] + \dots$  or the apparent solubility of *D*,  $D_t = D_s + ([DA] + [DA_2] + \dots) + 2([D_2A] + [D_2A_2] + \dots) + n[D_nA] +$

$[D_nA_2] + \dots$ . The higher terms are not normally significant at low dilutions for most systems. Then,  $D_t = D_s + [DA] + [DA_2]$ .

The equilibrium concentration of each solute species present in these mixtures follows the law of mass action. For the complex species we can write:

$$K_{\text{eq. } D_nA_m} = a_{D_nA_m}/(a_D)^n(a_A)^m \text{ or } K_{\text{eq. } DA}(a_D) = a_{DA}/a_A$$

$$K_{\text{eq. } DA_2}(a_D) = a_{DA_2}/(a_A)^2$$

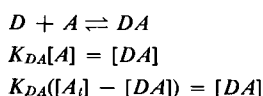
and  $K_{\text{eq. } D_2A}(a_D)^2 = a_{D_2A}/a_A$ , respectively

where  $K_{\text{eq.}}$  = the equilibrium constant;  $a$  = the activity. Since the Component  $D$  is kept saturated all the time, the dissolved  $D$  and solid  $D$  are in equilibrium, the activity of  $D$  in the solution is always constant, and in dilute solution the activity is approximately equal to the concentration, we can write:

$$K_{DA} = \frac{[DA]}{[A]}, \quad K_{DA_2} = \frac{[DA_2]}{[A]^2}, \quad \text{and} \quad K_{D_2A} = \frac{[D_2A]}{[A]} \quad (\text{Eq. 2})$$

where  $[DA]$ ,  $[DA_2]$ , and  $[D_2A]$  are the concentrations of the complex species  $DA$ ,  $DA_2$ , and  $D_2A$ , respectively.  $[A]$  is the concentration of free complexing agent, proton acceptor  $A$  in the system, and  $K_{DA}$ ,  $K_{DA_2}$ , etc., have been termed the interaction constants to distinguish them from the corresponding equilibrium constants ( $K_{\text{eq.}}$ ). It can be seen that for all complex formation which is first-order with respect to  $A$ , the linear relationship of  $D_nA = K_{D_nA}[A]$  can be derived.

If a 1:1 complex is assumed,



where  $[A]_t$  is the total concentration of the complexing agent added

$$K_{DA}[A]_t = [DA](1 + K_{DA})$$

$$[DA] = \frac{K_{DA}}{1 + K_{DA}} [A]_t$$

and from  $[D]_t = [DA] + [D_s]$

$$[D]_t = \frac{K_{DA}}{1 + K_{DA}} [A]_t + [D_s] \quad (\text{Eq. 3})$$

while  $[D]_t$  is the total concentration of  $D$  in the solution and  $[D_s]$  is the original solubility of  $D$ . Plots of total soluble  $D$  against the total amount of the proton acceptor added give a straight line relation. The slope of the line is equal to  $(K_{DA})/(1 + K_{DA})$ .

When a second-order complex, in respect to the complexing agent, is formed  $D + 2A \rightleftharpoons DA_2$  and  $K_{DA_2} = [DA_2]/[A]^2$ , which is a quadratic equation yielding a straight line plot of  $[DA_2]$  versus  $[A]^2$ , with the slope  $K_{DA_2}$ .

As previously mentioned, several forms of complex will theoretically exist in the solution, but the majority of  $D$  will be as  $DA$  or  $DA_2$  or a mixture of  $DA + DA_2$ .

Other minor complex species can be ignored and the total of  $D$  found in the solution can be assumed as:

$$D_t = [D_s] + [DA] + [DA_2] \quad (\text{Eq. 4})$$

and complexed  $D = [DA] + [DA_2]$ .

Since the exact amounts of  $DA$  and  $DA_2$  in a system will not be known, the data can be rationalized by assuming that all complexed  $D$  is in the form of the 1:1 type, and from Eq. 2 Eq. 4 is rewritten as

$$K_{1:1(\text{apparent})}[A] = K_{DA}[A] + K_{DA_2}[A]^2$$

$$K_{1:1(\text{apparent})} = K_{DA} + K_{DA_2}[A] \quad (\text{Eq. 5})$$

where  $K_{1:1(\text{apparent})}$  is the calculated interaction constant assuming all 1:1 type complexes.

From the above equation, the values of  $K_{DA}$  and  $K_{DA_2}$  can be derived as the intercept and the slope from the linear plot of  $K_{1:1(\text{apparent})}$  versus free complexing agent  $A$  in the system.

This solubility method has been used by Chulkaratana (37) to study hydrogen bond formation between salicylic acid, sub-

stituted phenols, and hydroquinone (proton donors) and acids, alcohols, esters, ketones, and lactones (proton acceptors).

**B. Liquid-Liquid Partition Method**—The Nernst distribution law (46, 47) can be defined as

$$[A]_0/[A]_w = e^{-(u_0^0 - u_w^0)/kT} = e^{-\Delta u^0/kT} = PC \quad (\text{Eq. 6})$$

where  $[A]_0$  and  $[A]_w$  are the equilibrium concentrations and  $u_0^0$  and  $u_w^0$  are the chemical potentials of solute  $A$  (in thermodynamic standard states) distributed between the two immiscible organic and aqueous phases.  $PC$  is the partition coefficient. Equation 6 holds only for the simplest cases in which the solute  $A$  in each phase is in the same state of aggregation. Deviations from this simple system often occur, however. Solute  $A$ , for example, may undergo association in the organic phase and/or dissociation in the aqueous phase. Problems relating to this have been discussed by many authors (48, 49). Furthermore there are several other factors which also affect the partition coefficient and which must be taken into account in any investigation involving the determination of partition coefficient.

(a) As the apparent partition coefficient will, in all cases, be the partition coefficient of the solute between two mutually saturated liquid mixtures, it is essential that the two liquid phases have minimal miscibility.

(b) The concentrations used should be as low as possible to minimize dimerization of solutes in the organic phase (50).

(c) Constant temperature should be maintained for all measurements.

(d) The system must be allowed to reach equilibrium. Hunter and Nash (51) and others (52) have given theoretical treatment on the rate of solute transfer between phases.

The partitioning of a hydrogen donor ( $D$ ) between two immiscible phases is influenced directly by the extent of complex formation in the apolar solvent when a hydrogen acceptor ( $A$ ) is present in the system. The complex formed ( $DA$ ) is normally extracted into the organic phase after equilibrium has been reached.

If the interaction is limited to only 1:1 species and occurs only in the organic phase then,



$$K_a^0 = \frac{a_{DA}}{a_D a_A} = \frac{[DA]\gamma_{DA}}{[D][A]\gamma_D \gamma_A} = K \frac{\gamma_{DA}}{\gamma_D \gamma_A} \quad (\text{Eq. 8})$$

where  $[A]$ ,  $[D]$ , and  $[DA]$  are the molecular concentrations of free  $A$ , free  $D$ , and complexed  $D$ , respectively,  $a$  and  $\gamma$  are activity and activity coefficient,  $K_a^0$  represents the complex stability constant in terms of activity, and  $K$  is the stability constant in terms of concentration. In most cases the  $\gamma$  terms are unknown and are assumed to be unity at low concentrations. If computation of  $K$  is based on  $D$ , then  $D$  in the organic phase is the sum of  $[D]$ ,  $[DA]$ , and perhaps some dimerized  $D$  species  $[D_2]$ . Thus,

$$[D]_t = [D] + [DA] + 2[D_2]$$

$$= [D] + [DA] + 2K_d[D]^2 \quad (\text{Eq. 9})$$

where  $K_d$  is the dimerization constant of  $D$  in the organic phase.

The complex concentration  $[DA]$  will be

$$[DA] = [D]_t - [D] - 2K_d[D]^2 \quad (\text{Eq. 10})$$

The  $[D]$  and  $[A]$  terms can be accurately determined from Eq. 6 by measuring  $D$  and  $A$  species in the aqueous phase. If, however, the  $A$  species is not soluble in the aqueous phase,  $[A]$  is obtained from  $[A]_t$  minus  $[DA]$  in the organic phase. The exact amount of  $D$  species in the aqueous phase can be obtained from the known  $pK_a$  of  $D$  and the pH of the aqueous phase.

For systems containing both 1:1 and 1:2 types of complex we have

$$[D]_t = [D] + [DA] + 2[D_2] + 2[D_2A] \quad (\text{Eq. 11})$$

and the complexes in terms of  $D$  will be

$$[DA] + 2[D_2A] = [D]_t - [D] - 2K_d[D]^2 \quad (\text{Eq. 12})$$

The observed stability constant,  $K_{\text{app}}$ , is then

$$K_{\text{app}} = \frac{[DA] + 2[D_2A]}{[D][A]} \quad (\text{Eq. 13})$$

**Table II**—Equilibrium Constants [ $l \cdot (\text{mole})^{-1}$ ] for Various Hydrogen-Bonding Interactions at 25°

Hydrogen Donor	$\sigma^e$	Solvent → Hydrogen Acceptor → Method → pKa <sup>f</sup>	Cyclohexane				Carbon Tetrachloride			
			TBP <sup>d</sup>		Sarin <sup>d</sup>		I-PTP		Griseofulvin <sup>b</sup>	
			LLP <sup>a</sup>	UV	LLP <sup>a</sup>	UV	ORD	ORD <sup>d</sup>	NMR <sup>c</sup>	ORD
1, Phenol	0.0	9.98	950	930	215		231	105	105	33
2, <i>p</i> -CH <sub>3</sub> -phenol	-0.171	10.14	640	620	130					
3, <i>p</i> -C <sub>2</sub> H <sub>5</sub> -phenol	-0.151	10.00	755		140					
4, <i>p</i> - <i>t</i> -C <sub>4</sub> H <sub>9</sub> -phenol	-0.197	10.25	575		100		49			
5, <i>p</i> -C <sub>6</sub> H <sub>5</sub> -phenol	+0.009	9.55	1380	1270	210					
6, <i>m</i> -OCH <sub>3</sub> -phenol	-0.115	9.65						125		35
7, <i>p</i> -OCH <sub>3</sub> -phenol	-0.268	10.21	712		135		247	69		23
8, <i>m</i> -F-phenol	+0.337	9.28		3320				283		84
9, <i>p</i> -F-phenol	-0.062	9.95	1900	1820	260		459	216		55
10, <i>m</i> -Cl-phenol	+0.373	9.13						418		109
11, <i>p</i> -Cl-phenol	+0.227	9.38	2840	2900	470		1801	273		87
12, <i>p</i> -Br-phenol	+0.232	9.36	2985		520		1145	296		89
13, <i>m</i> -I-phenol	+0.352	9.06						344		125
14, <i>p</i> -I-phenol	+0.276	9.31	3227		540		1696	268		82
15, 2,3,5,6-tetra-F-phenol	—	5.6			990			608		129
16, Penta-F-phenol	—	5.53			1720			950		265
17, <i>p</i> -COCH <sub>3</sub> -phenol	+0.874	—	6620							
18, <i>p</i> -COC <sub>2</sub> H <sub>5</sub> -phenol	—	—	4680		800					
19, <i>p</i> -CHO-phenol	+1.126	7.62	7782		1270					
20, <i>m</i> -CN-phenol	+0.678	8.61		9020	2300			1506		263
21, <i>p</i> -CN-phenol	+1.00	7.95	15550	17100	2150			1245		423
22, <i>p</i> -NO <sub>2</sub> -phenol	+1.270	7.15	20600		3000		33088	1925		830
23, Catechol	—	9.13	4950	5250	820		2328	569		
24, 4- <i>i</i> -C <sub>3</sub> H <sub>7</sub> -catechol	—	—	3440	3360	600					
25, 4-NO <sub>2</sub> -catechol	—	—	6210							
26, 1-Naphthol	—	9.85		1290	235	280				
27, 2-Naphthol	—	9.93		1130	240	270				

<sup>a</sup> LLP = liquid-liquid partition. <sup>b</sup> Reference 41. <sup>c</sup> Reference 59. <sup>d</sup> Reference 55. <sup>e</sup> Hammett  $\sigma$  values from References 41, 55. <sup>f</sup> References 41, 45.

$$\text{and } K_{\text{app}} = K_{1:1} + K_{1:1} \cdot 2K_{1:2} \cdot [D] \quad (\text{Eq. 14})$$

if we assume that  $DA + D = D_2A$  is acceptable. Then by plotting  $K_{\text{app}}$  against  $[D]$ ,  $K_{1:1}$  and  $K_{1:2}$  are obtained from the intercept and slope, respectively.

Details of some systems examined by the liquid-liquid partition are given in Table II.

**C. Spectropolarimetric Method [Optical Rotatory Dispersion (ORD)]**—The ORD method of studying hydrogen-bond complexes involves following the change in optical activity of a compound with change in wavelength of a polarized beam of light for the complexed and uncomplexed species. A full treatment of the theory and application of ORD has been given by Djerassi (53). The use of ORD to study hydrogen bond formation has been discussed by Meier and Higuchi (28).

Modification of spectrophotometric methods for the determination of equilibrium constants (34) from ORD data are not usually successful due to the fact that one measures a small difference between two rotations. A small variation in acceptor concentration (mainly through evaporation of the solvent) can lead to considerable error in the measured rotation. The calculation method for stability constants (34) enhances such errors so that at donor concentrations low enough to avoid dimerization the precision of the method is poor. In order to overcome these problems Meier and Higuchi (28) have developed an analysis method based on an equation of Rossotti and Rossotti (54). This has been used with success to study the camphor-phenol complex in carbon tetrachloride where open-chain phenol dimers are the donor species (28). ORD results for the interaction of phenols with griseofulvin (41) and levo-*S*-ethyl-*o*-ethylphenylphosphonothionate (*l*-PTP) (55) are given in Table II.

**D. Ultraviolet Absorption Method**—The UV absorption spectra of hydrogen-bonded species are different from those of the uncomplexed components, *i.e.*, the wavelength, slope, and intensity of the absorption bands change from the original unbonded species (56, 57) so that from suitable measurements the stability constant of a complex can be determined. The advantages of the UV method usually outweigh the disadvantages. A very low concentration ( $10^{-6}$ – $10^{-4}$  M) of the hydrogen donor is usually required so that the self-association problem can often be neglected. In addition, reproducible data can be obtained with ease, and with regard to the procedures concerned, this technique is simpler and more economic of time than those of solubility, partition, cryoscopy, NMR, *etc.*

The main disadvantage of the UV spectrophotometric technique is that this method does not permit convenient determination of the stoichiometry of complex formation. Usually a simple stoichiometry, *e.g.*, 1:1, is assumed in calculating the stability constants of these systems.

For computing the stability constants the equations reported by Meier and Higuchi (28) can be adapted. The assumptions involved are (a) only 1:1 complex is formed and (b) no association of the hydrogen donor or acceptor takes place. Thus for interaction between *D* and *A*,

$$A + D \rightleftharpoons DA, \quad K = [DA]/[D][A] \quad (\text{Eq. 15})$$

where  $[D]$  and  $[A]$  are the molar concentrations of free *D* and *A*, respectively. *K* is the 1:1 complex stability constant. If  $[D]_t$  and  $[A]_t$  denote the total concentrations of *D* and *A* then

$$[A] = [A]_t - [DA] \quad (\text{Eq. 16})$$

$$[D] = [D]_t - [DA] \quad (\text{Eq. 17})$$

from Eq. 15

$$[DA] = K([A]_t[D]_t - [A]_t[DA] - [D]_t[DA]^2) = \frac{[D]_t[A]_t}{1/K + [D]_t + [A]_t - [DA]} \quad (\text{Eq. 18})$$

which is equal to

$$[DA] = \frac{[D]_t[A]_t + [DA]^2}{1/K + [D]_t + [A]_t} \quad (\text{Eq. 19})$$

The observed optical density (absorbance), *B*, of the system is the sum of the absorbances of *D*, *A*, and *DA* species (Beer's law).

Accordingly,

$$B = (\epsilon_D \cdot l \cdot [D]) + (\epsilon_A \cdot l \cdot [A]) + (\epsilon_{DA} \cdot l \cdot [DA]) \quad (\text{Eq. 20})$$

where  $\epsilon_D$ ,  $\epsilon_A$ ,  $\epsilon_{DA}$  are the molecular absorptivities of *D*, *A*, and *DA*, respectively and *l* is the cell length. The difference in absorbance of

the mixture from that of the total  $D$  and  $A$  species is given by

$$\Delta B = (B - B_D - B_A) = \epsilon_D \cdot l \cdot ([D]_t - [DA]) + \epsilon_A \cdot l \cdot ([A]_t - [DA]) + \epsilon_{DA} \cdot l \cdot [DA] - \epsilon_D \cdot l \cdot [D]_t - \epsilon_A \cdot l \cdot [A]_t = [DA] \cdot l \cdot (\Delta\epsilon) \quad (\text{Eq. 21})$$

where

$$\Delta\epsilon = \epsilon_{DA} - \epsilon_A - \epsilon_D \quad (\text{Eq. 22})$$

If in the system under investigation,  $A$  does not absorb then  $\Delta B = B - B_A$ , and  $\Delta\epsilon = \epsilon_{DA} - \epsilon_D$ . In combining Eqs. 19 and 21 the following Eq. 23 is then obtained if we assume that  $[DA]^2 \ll ([D]_t[A]_t)$  and can therefore be neglected in Eq. 19. Thus,

$$\frac{l [D]_t [A]_t}{\Delta B} = \frac{[D]_t + [A]_t}{\Delta\epsilon} + \frac{1}{K \Delta\epsilon} \quad (\text{Eq. 23})$$

By plotting the left-hand side of Eq. 23 against known values of  $([D]_t + [A]_t)$ ,  $K$  can be obtained from the intercept and slope. The  $K$  values obtained in this manner are only approximate values because of the above assumption, *i.e.*,  $[DA]^2$  may not be negligible. If Eqs. 18 or 19 are rearranged without neglecting the  $[DA]^2$  term,

$$[DA]^2 - [DA]([A]_t + [D]_t + 1/K) + ([A]_t[D]_t) = 0 \quad (\text{Eq. 24})$$

and

$$[DA] = \frac{([A]_t + [D]_t + 1/K) - \sqrt{([A]_t + [D]_t + 1/K)^2 - 4([D]_t[A]_t)}}{2} \quad (\text{Eq. 25})$$

The above quadratic form of Eq. 25 is useful in computing the complex concentrations from the known values of  $[A]_t + [D]_t$  and the preliminary  $K$  obtained from Eq. 23. Similarly if we combine Eq. 19 and 21 without neglecting the  $[DA]^2$  term the exact Eq. 26 is obtained

$$\frac{l([D]_t[A]_t + [DA]^2)}{\Delta B} = \frac{[D]_t + [A]_t}{\Delta\epsilon} + \frac{1}{K \Delta\epsilon} \quad (\text{Eq. 26})$$

Thus the complex stability constant from Eq. 26 should represent a better  $K$  value than that obtained from Eq. 23. The procedure involves successive approximations, *i.e.*, the initial  $K$  and  $[DA]$  are calculated from Eqs. 23 and 25. Then from Eq. 26 the new  $K$  value is obtained. By repeating the same computations using Eqs. 25 and 26 successively, constant values of  $[DA]$  and  $K$  are eventually obtained which should represent the true  $K$  value of the system under investigation.

The correct concentration scale to be used when analyzing spectroscopic data by methods similar to the above has been discussed by Kuntz *et al.* (58). They concluded that the scale appropriate to a given system could usually be determined experimentally but from the limited data available molarity appeared to be a better concentration unit than either mole fraction or molality.

Some results obtained with UV for the interaction of phenols with tri-*n*-butylphosphate (TBP) and sarin (isopropyl methylphosphonofluoridate) (55) are given in Table II.

**Analysis of Hydrogen-Bonding Data**—The quantitative analysis of hydrogen-bonding data is directly applicable to (a) the prediction of useful association constants; (b) the detection of deviations shown by specific systems from the expected behavior; and (c) in providing a means of storing the ever-increasing amount of data in a more condensed manner.

Since any method of storing information should try to reduce the data to a minimum number of parameters an attempt is made to explain the data on hydrogen bonding in terms of one parameter for each hydrogen accepting molecule and one parameter for each hydrogen donating molecule.

**A. Simple Relationship**—An initial attempt can be made to establish whether association constants ( $K_{DA}$ ) can be adequately described in terms of a simple relationship expressed by the following equation

$$K_{DA} = K_D K_A \quad (\text{Eq. 27})$$

while  $K_A$  and  $K_D$  are parameters related to the bonding availabilities

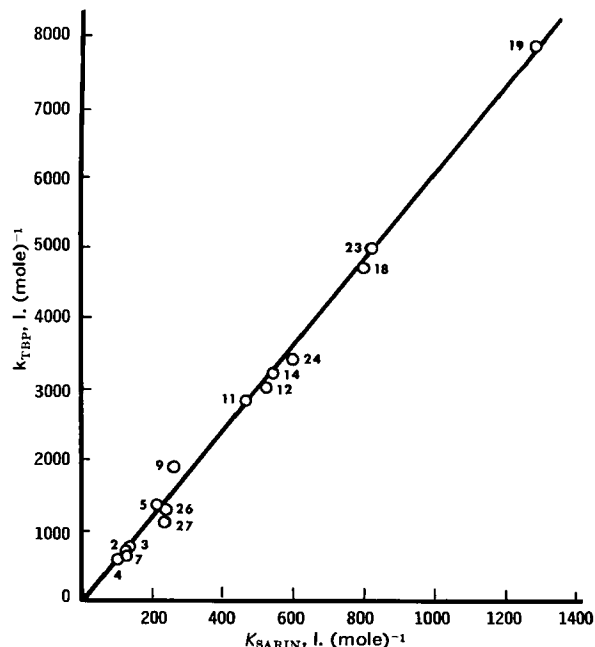


Figure 1—A plot showing the linear relationship between equilibrium constants of complexes formed by TBP and sarin with a series of phenolic compounds in cyclohexane at 25°. The phenolic compounds are numbered as in Table II.

of the hydrogen acceptor and donor, respectively. An initial analysis appears to support the existence of this relationship, since plots of the  $K_{DA}$  values for the interaction of a given hydrogen acceptor with various phenolic hydrogen donors against the  $K_{DA}$  values for the interaction of a second acceptor with the same donors (data from Table II) give linear plots which pass through the origin. An example is shown in Fig. 1.

By assigning an arbitrary figure to the  $K_D$  value of phenol ( $K_D = 10$ ) it is possible to obtain  $K_A$  values for a few hydrogen acceptors, including TBP and sarin, and  $K_D$  values for several substituted phenols. A good agreement between the  $K_D$  values of these phenols derived from different series of interactions is obtained, again indicating the applicability of Eq. 27 to the hydrogen-bonding data.

When attempts are made to extend the application of the above equation to systems involving nonphenolic hydrogen donors, it is found that plots of  $K_{DA}$  values for the interaction of two given hydrogen donors with a similar series of acceptors, or for the interaction of two given hydrogen acceptors with a similar series of hydrogen donors, do not always give linear slopes which pass through the origin. It would appear, therefore, that the simple relationship expressed by the above equation may be limited in its application to systems involving a series of chemically similar hydrogen donors or acceptors. This limitation appears to parallel that observed by Gheretti and Lusa (60) from their investigations on the relationship between  $\Delta H$  and  $\Delta\nu_{OH}$  values associated with hydrogen-bonding interactions of a series of phenols with different acceptors.  $\Delta H$  is the enthalpy of bonding and  $\Delta\nu_{OH}$  the proton donor wave number displacement from spectral analysis. They found that  $\Delta\nu_{OH}$  values may be used to predict  $\Delta H$  values only for a given acceptor and a very homogeneous series of hydrogen donors and tentatively suggested that the substituent effects in substituted phenols were themselves affected by the strength of the acceptors.

**B. Linear Free Energy Relationships**—The empirical correlations that have been used in connection with the rates of chemical reactions and their equilibria by Hammett *et al.* (61–63) have generally taken a form of a linear relationship between the logarithms of the rate or equilibrium constants for two reactions, which have been subjected to the same variations in reaction conditions. Such a relationship is expressed by the equation

$$\log K = m \cdot \log K' + c \quad (\text{Eq. 28})$$

where  $K$  and  $K'$  are the corresponding rate or equilibrium constants

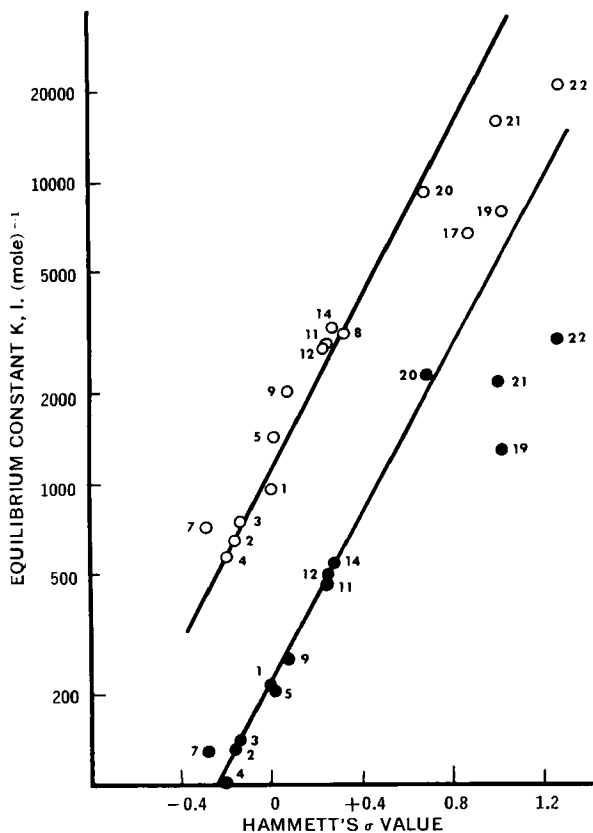


Figure 2—A plot showing the approximate relationship between the equilibrium constant of TBP (O) and sarin (●) phenol complexes and the Hammett  $\sigma$  values of the phenolic compounds as listed in Table II.

and  $m$  and  $c$  are the slope and intercept of the plot of  $\log K$  versus  $\log K'$ .

Since the logarithms of equilibrium and rate constants are proportional to the standard free energy change associated with the reaction ( $\Delta F^0$ ) and the standard free energy of activation ( $\Delta F^*$ ), respectively, it follows that Eq. 28 indicates a linear relationship between standard free energy changes, *i.e.*,

$$\Delta F = m \Delta F' + c \quad (\text{Eq. 29})$$

The standard free energy change ( $\Delta F^A$ ) of a Reaction  $A$  may be considered to be a function of a number of independent Variables  $x$ ,  $y$ , *etc.* The variation of the standard free energy change at constant temperature can then be expressed by

$$d\Delta F^A = \left(\frac{\delta\Delta F^A}{\delta x}\right)_T \cdot dx + \left(\frac{\delta\Delta F^A}{\delta Y}\right)_T \cdot dy + \dots \quad (\text{Eq. 30})$$

For a finite change in the Variable  $x$  from some arbitrary standard value  $x_0$  to  $x_1$  the free energy change will be given by

$$\Delta F_1^A - \Delta F_0^A = \left(\frac{\delta\Delta F^A}{\delta x}\right)_T (x_1 - x_0) \quad (\text{Eq. 31})$$

provided that the other variables are maintained constant and that  $\left(\frac{\delta\Delta F^A}{\delta x}\right)_T$  also remains constant within the variation of  $x$ .

Since the equilibrium constant  $K$  of a reaction can be related to the standard free energy change accompanying the reaction by the expression  $\Delta F = -2.303 RT \log K$ , then Eq. 31 can be written

$$\log \left(\frac{K_1}{K_0}\right)_A = \frac{(\delta\Delta F^A/\delta x)_T \cdot (x_0 - x_1)}{2.303 RT} \quad (\text{Eq. 32})$$

Similarly, the effect of the same change in the Variable  $x$  on another Reaction  $B$  can be expressed by Eq. 33

$$\log \left(\frac{K_1}{K_0}\right)_B = \frac{(\delta\Delta F^B/\delta x)_T \cdot (x_0 - x_1)}{2.303 RT} \quad (\text{Eq. 33})$$

The combination of Eqs. 32 and 33 yields Eq. 34

$$\log \left(\frac{K_1}{K_0}\right)_B = \log \left(\frac{K_1}{K_0}\right)_A \left(\frac{\delta\Delta F^B}{\delta x}\right)_T / \left(\frac{\delta\Delta F^A}{\delta x}\right)_T \quad (\text{Eq. 34})$$

which indicates that the effect of the change in the Variable  $x$  on Reaction  $B$  may be considered to be composed of two parts, *i.e.*, on  $\log (K_1/K_0)_A$ , which depends solely on the change in the Variable  $x$ , and on  $(\delta\Delta F^B/\delta x)_T/(\delta\Delta F^A/\delta x)_T$  which depends on the susceptibility of Reaction  $B$  to changes in  $x$  relative to Reaction  $A$ , and on the reaction conditions.

The relationship given by Eq. 34 has been applied to many types of chemical reactions and has provided much information on the effects of substituents and changes in reaction conditions (63, 64).

The linear free energy equation given by Hammett (61) and modified by Taft (64) has been applied by Lawrence and Wojtkowiak (65, 66), Nakano (41), and Hou (55) to the hydrogen-bonding data of substituted phenol complexes

$$\log (k/k_0) = \rho\sigma \quad (\text{Eq. 35})$$

where  $k$ ,  $k_0$ , are the reaction (rate) constants of the substituted and unsubstituted species,  $\rho$  is a reaction constant, and  $\sigma$  a substituent constant. Plots of  $\Delta\nu_{OH}$  (65, 66) or  $\log K$  (41) against the Hammett  $\sigma$  or Taft  $\sigma^0$  give approximate correlations (Fig. 2). Nakano (41) and Hou (55) have also obtained a linear relationship between  $\log K$  and  $pK_a$  of substituted phenols for griseofulvin-phenol complexes and organo-phosphorus compound-phenol complexes (Fig. 3).

In the case of hydrogen-bonding interactions, however, the only mechanism that is involved is the formation of the hydrogen bond between a molecule possessing a suitable hydrogen atom and a molecule possessing a suitable electronegative hydrogen-accepting center. The change in free energy that accompanies such bond formation will depend on the hydrogen-donating and hydrogen-accepting abilities of the respective molecules, and these abilities will, in turn, depend on the structures of the two molecules. Thus, it would seem feasible to expect that hydrogen-bonding interactions should also be capable of correlation by an expression similar to Eq. 34 in which one term on the right-hand side of the

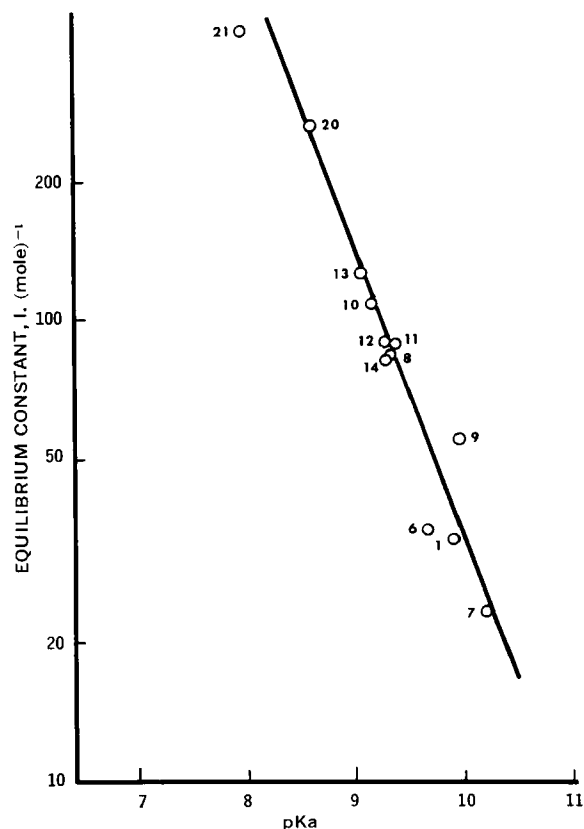


Figure 3—A plot of equilibrium constant against  $pK_a$  for the interaction of griseofulvin with substituted phenols in carbon tetrachloride at 25°. The numbers correspond to the phenols in Table II.

**Table III**— $h_A$  and  $\log K_{DA_0(\text{calc.})}$  Values for Hydrogen-Bonding Interactions in Saturated Hydrocarbon Solution at 25°

H-Acceptor	Solvent	$h_A$	$s^a$	$r^b$	$n^c$	$\log K_{DA_0(\text{calc.})}^d$
TBP <sup>e</sup>	Cyclohexane	1.00	—	—	—	—
Sarin <sup>e</sup>	Cyclohexane	0.90	0.073	0.986	18	2.25
<i>l</i> -PTP <sup>e</sup>	Cyclohexane	1.63	0.179	0.978	9	2.30
(CH <sub>3</sub> ) <sub>3</sub> N <sup>f</sup>	Cyclohexane	0.74	0.022	0.996	4	1.94
CH <sub>3</sub> ·COOCH <sub>3</sub> <sup>g</sup>	<i>n</i> -Heptane	0.35	0	1.000	4	1.08

<sup>a</sup>  $s$  = SD of experimental measurements from the regression line. <sup>b</sup>  $r$  = correlation coefficients. <sup>c</sup>  $n$  = number of interactions involved in the calculation of  $h_A$ . <sup>d</sup>  $\log K_{DA_0(\text{calc.})}$  = intercept of regression line with ordinate ( $h_D = 0$ ). <sup>e</sup> Calculations based on results given in Table II. <sup>f</sup> Calculations based on results given in Reference 39. <sup>g</sup> Calculations based on results given in Reference 40.

equation provides a measure of the effect of the change in structure of the hydrogen donor and the other term provides a measure of the effect of structural change in the hydrogen acceptor. In its application to hydrogen-bonding systems Eq. 34 may therefore be written as

$$\log \left( \frac{K_1}{K_0} \right)_A = h_D \cdot h_A \quad (\text{Eq. 36})$$

where  $K_1$  and  $K_0$  are the equilibrium constants for the separate interactions of two hydrogen donors with a common hydrogen acceptor ( $A$ ),  $h_D$  is a measure of the effect of change in the structure of the hydrogen donor on the interaction and is equal to  $\log (K_1/K_0)_{A_0}$ , where  $K_1$  and  $K_0$  are the equilibrium constants for the interaction of the previous two donors with a hydrogen acceptor ( $A_0$ ), which is chosen as an arbitrary standard, and  $h_A$  is a measure of the effect of the acceptor  $A$  on the hydrogen bonding equilibrium relative to that of  $A_0$ .

The above definitions of  $h_A$  and  $h_D$  imply that the solvent does not affect the association between the two solute molecules. The possible mechanisms of solvent interference will include both general and specific interactions between the solvent and either of the two solutes or the complex, although the degree of deviation produced by general solvent effects (e.g., changes in the dielectric constant) will probably be much smaller than that produced by specific effects such as the formation of hydrogen bonds between the solvent and any of the other species present.

From Eq. 36 and the definition of  $h_D$  given above, it can be seen that in the case of interactions involving the arbitrarily chosen standard hydrogen acceptor  $A_0$ , the value of  $h_A$  is one. It is also necessary to choose a standard hydrogen donor in order that the  $h_D$  values may also be compared in a quantitative manner and again from the definition of  $h_D$  it may be seen that the  $h_D$  value for this standard is zero.

As previously stated, Eqs. 34 and 36 will only be useful if solvent effects, especially those of a specific nature, can be neglected. Because of this limitation it is necessary to specify that the solvent used for the reference interaction should be inert, and saturated hydrocarbons such as hexane, heptane, and cyclohexane have been chosen as being satisfactory in this respect. It is realized that even changes in saturated hydrocarbon solvents may affect the hydrogen-bonding equilibria to some extent but in view of the small number of systematic studies (41) carried out in these solvents it does not seem possible to take these relatively small variations into account in the present analysis. In addition, since hydrogen-bonding interactions are sensitive to temperature changes, it is necessary to specify a fixed temperature for the reference interaction. A value of 25° has been chosen since most investigations have been made either at this temperature or over a range that includes this value.

Phenol has been chosen to represent the reference hydrogen donor (i.e.,  $h_D = 0$ ), because it is a strong donor and, together with many of its substituted forms, it has been involved in many of the more recent and more reliable investigations on hydrogen bonding with a wide variety of acceptors. The choice of a reference hydrogen acceptor is not so easy to make since the number of investigations involving a common hydrogen acceptor in an inert solvent is somewhat limited. The acceptors that have been used for interaction with a number of hydrogen donors in saturated hydrocarbon solvents are *l*-PTP, TBP, sarin (Table II), methylacetate (39), and trimethylamine (40). If the measurements in systems in which the hydrogen donors may be expected to show steric effects are discounted, then the available number of equilibria involving methylacetate or trimethylamine is low and the utility of these compounds

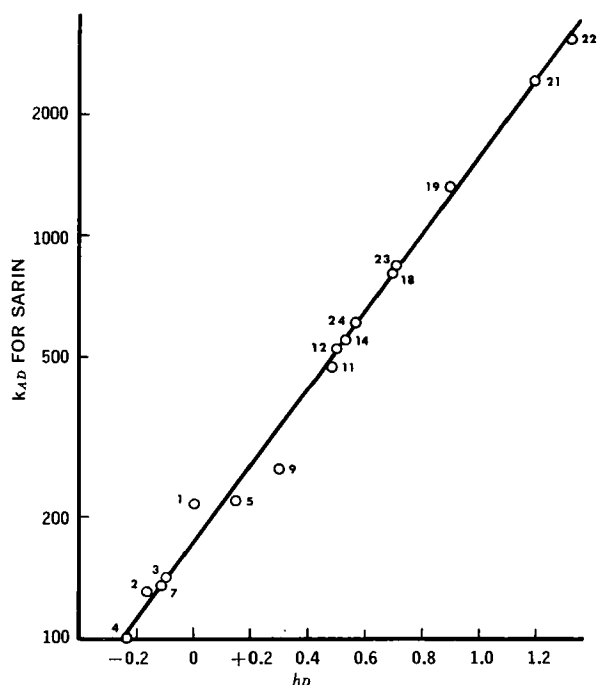
as the reference acceptor is therefore low. In the case of the remaining systems, only the series involving TBP has received any cross-checking of the values of the equilibrium constants by more than one method of measurement. For this reason, TBP has been selected as the reference hydrogen acceptor, for which  $h_A = 1$ .

Table III shows the  $h_A$  values and  $\log K_{DA_0}$  values for sarin, *l*-PTP, trimethylamine, and methylacetate in saturated hydrocarbon solution at 25°. These values were obtained from plots of  $\log K_{DA}$  for each of these series (Table II) versus the  $h_D$  values derived from the TBP series, by a correlation method similar to that outlined by Jaffé (62) (Fig. 4).

The values of  $h_D$  shown in Table IV were calculated from the results for each series by using Eq. 37

$$h_D = \bar{h}_D - b(\bar{Y} - Y) \quad (\text{Eq. 37})$$

where  $b = r^2/h_A$  and  $Y = \log K_{DA}$ . A comparison of the  $h_D$  values obtained for the same hydrogen donor from the different series indicates a reasonable agreement. A list of preferred  $h_D$  values is also shown in Table IV. This list is based on the average or weighted average of the available results. It is realized that such a procedure makes the values dependent on the data available at any one time, and may also obscure some deviations. However, such disadvantages may only be overcome by the availability of a suitable series of results which could be used to derive a set of "primary"  $h_D$  values. Although the TBP series appears to be the most suitable that is available at present, it is still subject to the disadvantages that (a) only some of the equilibrium constants for this series have been crosschecked by two methods of determination and the differences between crosschecked values show an upper limit of approximately 6%; and (b) the TBP molecule has more than one electro-



**Figure 4**—A plot of  $\log K_{AD}$  for sarin against the  $h_D$  values derived for this series from the TBP series using eq. 36.

**Table IV**— $h_D$  Values for Hydrogen Donors in Saturated Hydrocarbon Solvents at 25°

Hydrogen Acceptor → Hydrogen Donor	Solvent →				<i>n</i> -Heptane CH <sub>3</sub> -COOCH <sub>3</sub> <sup>c</sup>	"Preferred" Values
	TBP <sup>a</sup>	Sarin <sup>a</sup>	Cyclohexane (CH <sub>2</sub> ) <sub>6</sub> N <sup>b</sup>	<i>l</i> -PTP <sup>a</sup>		
Phenol	0	0	0	0	0	
<i>p</i> -CH <sub>3</sub> -phenol	-0.17	-0.14	-0.17		-0.16	-0.16
<i>p</i> -C <sub>2</sub> H <sub>5</sub> -phenol	-0.10	-0.11				-0.11
<i>p</i> - <i>t</i> -C <sub>4</sub> H <sub>9</sub> -phenol	-0.21	-0.27		-0.34		-0.27
<i>p</i> -C <sub>6</sub> H <sub>5</sub> -phenol	0.15	0.09				
<i>p</i> -OCH <sub>3</sub> -phenol	-0.12	-0.12		0.07		-0.12
<i>p</i> -F-phenol	0.30	0.19		0.23		0.24
<i>p</i> -Cl-phenol	0.49	0.47	0.49	0.58	0.48	0.48
<i>p</i> -Br-phenol	0.50	0.51		0.46		0.49
<i>p</i> -I-phenol	0.54	0.53		0.56		0.54
2,3,5,6-Tetra-F-phenol		0.03				
Penta-F-phenol		0.29				
<i>p</i> -COCH <sub>3</sub> -phenol	0.85					
<i>p</i> -COC <sub>2</sub> H <sub>5</sub> -phenol	0.70	0.72				0.71
<i>p</i> -CHO-phenol	0.92	0.93				0.93
<i>m</i> -CN-phenol	0.98	1.21				
<i>p</i> -NO <sub>2</sub> -phenol	1.24	1.18				1.21
<i>p</i> -NO <sub>2</sub> -phenol	1.34	1.34		1.32		1.33
Catechol	0.74	0.73		0.64		0.74
4- <i>t</i> -C <sub>3</sub> H <sub>7</sub> -catechol	0.56	0.58				0.57
4-NO <sub>2</sub> -catechol	0.82					
1-Naphthol	0.14	0.18	0.14		0.17	0.16
2-Naphthol	0.08	0.18	0.57		0.26	0.17
Triphenylmethanol			-1.15			
<i>n</i> -Heptanol			-1.87			
2,5-Dimethylpyrrole			-1.87			
Indole			-1.32			

<sup>a</sup> Calculations based on results given in Table II. <sup>b</sup> Calculations based on results given in Reference 39. <sup>c</sup> Calculations based on results given in Reference 40.

negative center capable of associating with a hydrogen donor. Although the intermolecular association is only likely to involve the coordinated oxygen atom of the phosphate group, the presence of the remaining oxygen atoms detract from the ideality of TBP as the reference hydrogen acceptor.

*C. Solvent Effects*—The values of stability constants of complexes formed by hydrogen bonding are dependent upon the solvent in which the measurements are made. The interactions that occur between solutes and solvents can, in general, be divided into two types. Strong specific interactions, usually of the donor-acceptor

**Table V**— $h_A$ ,  $h_D$ , and  $\log K_{\text{calc.}}$  Values for Hydrogen-Bonding Interactions in Carbon Tetrachloride Solutions at 25°

H-Acceptor → Solvent →	<i>l</i> -PTP <sup>a</sup> CCl <sub>4</sub>	Griseofulvin <sup>b</sup> CCl <sub>4</sub>	Quinoline- 1-oxide <sup>c</sup> CCl <sub>4</sub>	Pyridine <sup>d</sup> CCl <sub>4</sub>	DMSO <sup>e</sup> C <sub>2</sub> Cl <sub>4</sub>
$h_A$	0.94	1.01	0.74	0.73	0.94
$s^f$	0.053	0.071	0.063	0.033	0.029
$r^g$	0.994	0.992	0.985	0.994	0.999
$n^h$	9	8	3	6	5
$\log K_{\text{calc.}}$ <sup>i</sup>	2.00	1.48	2.24	1.75	1.85

H-Donor	$h_D$ values				Average $h_D$ Value
Phenol	0				
<i>p</i> -CH <sub>3</sub> -phenol			-0.11	-0.18	-0.14
<i>p</i> - <i>t</i> -C <sub>4</sub> H <sub>9</sub> -phenol				-0.22	-0.22
<i>p</i> -OCH <sub>3</sub> -phenol	-0.25	-0.10		-0.18	-0.17
<i>p</i> -F-phenol	0.33	0.27			0.30
<i>p</i> -Cl-phenol	0.46	0.46	0.50	0.47	0.47
<i>p</i> -Br-phenol	0.49	0.47			0.48
<i>p</i> -I-phenol	0.44	0.43		0.54	0.47
<i>p</i> -NO <sub>2</sub> -phenol	1.44	1.42			1.34
<i>p</i> -CN-phenol	1.23	1.13			1.18
<i>m</i> -CN-phenol	1.32	0.93			
Catechol	0.83				0.83
<i>m</i> -CH <sub>3</sub> -phenol			-0.14	-0.16	-0.15
<i>m</i> -OCH <sub>3</sub> -phenol	0.03	0.07			0.05
<i>m</i> -F-phenol	0.47	0.44			0.46
<i>m</i> -Cl-phenol	0.67	0.56	0.71	0.66	0.65
<i>m</i> -I-phenol	0.57	0.62			0.60
2,3,5,6-Tetra-F-phenol	0.85	0.63			0.74
Penta-F-phenol	0.99	0.93			0.96
<i>m</i> -NO <sub>2</sub> -phenol					1.17

<sup>a</sup> Calculations based on results given in Table II. <sup>b</sup> Calculations based on results given in Reference 80. <sup>c</sup> Calculations based on results given in Reference 81. <sup>d</sup> Calculations based on results given in Reference 82. <sup>e</sup> Calculations based on results given in Reference 60. <sup>f</sup>  $s$  = SD of experimental measurements from the regression line. <sup>g</sup>  $r$  = correlation coefficient. <sup>h</sup>  $n$  = number of interactions involved in the calculation of  $h_A$ . <sup>i</sup>  $\log K_{\text{calc.}}$  = intercept of regression line with ordinate ( $h_D = 0$ ).



Table VI—The Equilibrium Constants for the Association of Carbon Tetrachloride with Phenol ( $K_{DS}$ ) and with Various Bases  $K_{AS}$  at 25°

H-Donor	H-Acceptor	$K_{DA}$ (Hydrocarbon)	Ref.	$K_{DA}'$ ( $\text{CCl}_4$ )	Ref.	$K_{DS}$	$K_{AS}$
Phenol	Benzene	0.48	83	0.31	84	0.05	0
Phenol	Dioxane	19	83	8.5	85	0.12	0.05
Phenol	Isophorone	70	77	30	77	0.13	0.05
Phenol	Pyridine	84	42, 86	45	42	0.08	0.02
Phenol	$(\text{C}_2\text{H}_5)_3\text{N}$	85	41, 85	58	87	0.05	0
Phenol	$\text{CH}_3\text{—COOC}_2\text{H}_5$	10.1	83	9.2	31, 88	0.01	—
Phenol	$(\text{C}_2\text{H}_5)_3\text{P} \rightarrow \text{O}$	9402	89	2512	35	0.26	0.14
Phenol	TBP	940	56	276	56	0.23	0.12
Phenol	DMA	295	73	136	73, 77	0.12	0.04

type, result in molecular complexes with well-defined stoichiometries and geometries. The weaker nonspecific interactions are thought to arise from the differences in the bulk properties (e.g., dielectric constants) of the solvents. The contributions of these different mechanisms to the net effect have received a considerable amount of discussion (67–74) and disagreement still exists, especially in those systems involving nonpolar or weakly polar solvents. The formation of hydrogen-bonded solute-solvent complexes has been suggested even in such solvents. For example, the effects of carbon tetrachloride and carbon disulfide on hydrogen donor solvents have been ascribed to the formation of weak hydrogen bonds involving the  $\delta^-$ - $\delta^+$  and  $\delta^-$ - $\delta^+$  dipoles, respectively (75),



and the interaction of the  $\pi$  electron systems of aromatic hydrocarbons and ethylenic linkages is well documented. In addition, chloroform is capable of forming weak hydrogen bonds with acceptor molecules. Although other mechanisms for specific interactions are possible [e.g., the dipole-induced dipole interactions between triethylphosphine oxide and carbon tetrachloride suggested by Gramstad (76)], the extent of such interaction is influenced by the same factors which influence the ability of the solute to act as a hydrogen donor or acceptor.

Recently, Nakano *et al.* (77) have developed a new iterative method of calculating stability constants from proton magnetic resonance data. This has enabled them to obtain precise data on the effect of various solvents for the interaction of phenol with dimethyl acetamide (DMA) and various ketones. In general, the value of the stability constant was much higher for saturated hydrocarbons than for nonhydrocarbon solvents. This was thought to be due to specific interactions between the solute and the latter solvents as had been pointed out by earlier investigators (25, 35, 42). From purely entropic considerations a direct relationship between stability constant and molar volume of the hydrocarbon solvents was expected. However, only a slight trend in this direction was apparent. Solvent effects for the phenol-DMA system have also been studied by Takahashi *et al.* (73) who found that the stability constant varied from 295 l. (mole)<sup>-1</sup> in cyclohexane to 130 l. (mole)<sup>-1</sup> in carbon tetrachloride and 16 l. (mole)<sup>-1</sup> in chloroform. For a chloroform-carbon tetrachloride mixed solvent the equilibrium constant ( $K$ ) varied linearly with  $K[\text{CHCl}_3]$  due to the formation of a 1:1 DMA-chloroform complex.

Hou (55) has found that for relatively nonpolar solvents the stability constant for the phenol-TBP complex decreased with increase in dielectric constant. The most probable solvent interaction was believed to be due to solvent-hydrogen donor interaction to form weak hydrogen bonds thus competing with the hydrogen acceptor rather than to some dielectric effect. The importance of solvation in hydrogen bonding interactions has been discussed recently by Barriol and Weisbecker (78).

At the present time the lack of any systematic determinations of equilibrium constants for hydrogen-bonding interactions in solvents which may interfere with the equilibrium precludes a quantitative analysis of solvent effects, except in the case of carbon tetrachloride, which is commonly used in IR spectroscopic measurements.

Two methods of treating the data in carbon tetrachloride solution are given below.

**Incorporation of Solvent Effects in  $h_A$  Values**—It would seem feasible to expect that a solvent which is capable of undergoing a specific interaction with either the hydrogen-donating solute or the hydrogen-accepting solute should have an effect on the  $h_A$  or

$h_D$  values, respectively. In many cases the solvent may be capable of interaction with both types of compound, thus giving rise to simultaneous variation in  $h_A$  and  $h_D$ . Carbon tetrachloride appears to be an example of this latter class, since in addition to its ability to form weak bonds with hydrogen donors, as previously mentioned, it appears to interact with hydrogen acceptors such as pyridine (79) and organophosphorus compounds (76). In spite of the dual type of interaction associated with carbon tetrachloride, an analysis of equilibrium constants involving the inclusion of all solvent effects in the  $h_A$  values provides results for  $h_D$  values which show reasonable agreement with those obtained from measurements in saturated hydrocarbon solvents. These results, which are shown in Table V, were derived from plots of  $\log K_{DA}$  in carbon tetrachloride versus the preferred  $h_D$  values shown in Table IV.

**Calculation of the Equilibrium Constants for the Interaction of Carbon Tetrachloride with Hydrogen-Accepting and Hydrogen Donating Solutes**—The interaction of the solvent with one or both components involved in an equilibrium will result in decrease in the equilibrium constant relative to the value obtained in an inert solvent. It can be shown (42) that the equilibrium constants in the two solvents can be related by Eq. 38

$$K_{DA} = K_{DA}'(1 + SK_{AS})(1 + SK_{DS}) \quad (\text{Eq. 38})$$

where  $K_{DA}$  and  $K_{DA}'$  are the equilibrium constants for the association between the hydrogen-donating and hydrogen-accepting solutes in the inert solvent and interfering solvent, respectively.  $K_{AS}$  and  $K_{DS}$  are the respective equilibrium constants for the association of the interactants with the solvent, and  $S$  is the molar concentration of the solvent. If it is assumed that the interaction between the solvent and the hydrogen-accepting solute is negligible (i.e.,  $K_{AS} = 0$ ) then  $K_{DS}$  is given by Eq. 39

$$K_{DS} = \frac{1}{S} \left( \frac{K_{DA}}{K_{DA}'} - 1 \right) \quad (\text{Eq. 39})$$

The values of  $K_{DS}$  obtained from Eq. 39 using the equilibrium constants for the association of phenol with a series of bases in inert solvents and carbon tetrachloride are shown in Table VI. The variation in the  $K_{DS}$  values suggests that the earlier assumption of negligible carbon tetrachloride-hydrogen acceptor interaction is invalid and that such interaction is appreciable in the case of triethylphosphine oxide and TBP. If a value of 0.05 l. (mole)<sup>-1</sup> is taken as a reasonable approximation for  $K_{DS}$  then the values of  $K_{AS}$  for the association of phenol with the various bases are as shown in Table VI.

Unfortunately, the equilibrium constants for many of the hydrogen-bonding systems in carbon tetrachloride solution reported by Gramstad *et al.* (25, 34, 35, 90, 95) cannot be utilized in a quantitative treatment of solvent effects because the equilibrium constants for the same systems in an inert solvent are not yet available. Gramstad's results, however, may be used to show that the relationship expressed by Eq. 36 is not restricted in its application solely to phenolic hydrogen donors. By using the interaction between triethylphosphine oxide and phenol in carbon tetrachloride at 20°, as the reference interaction (i.e.,  $h_A^*$  for triethylphosphine oxide = 1, and  $h_D^*$  values for phenol = 0)<sup>1</sup> it is possible to calculate

<sup>1</sup>  $h_A^*$  and  $h_D^*$  symbols are used in order to indicate that these series of values are based on a different reference interaction from that used in the calculation of  $h_A$  and  $h_D$  values.

Table VII— $h_A^*$  and  $h_D^*$  Values for Various Hydrogen Donors in  $\text{CCl}_4$  at  $20^\circ$

H-Acceptor	$(\text{C}_2\text{H}_5)_3\text{PO}$	$(\text{C}_6\text{H}_5)_3\text{PO}$	$\text{C}_5\text{H}_5\text{N}$	$\text{N}(\text{C}_2\text{H}_5)_3$	$(\text{CH}_3)_3\text{PO}_4$	$\text{CCl}_3\text{P}(\text{O})\text{-(OC}_2\text{H}_5)_2$	$\text{HP}(\text{O})\text{-(OC}_2\text{H}_5)_2$	$(\text{C}_2\text{H}_5)_2\text{N-P}(\text{O})\text{-(OC}_2\text{H}_5)_2$
$h_A^*$ Value	1	0.88	0.83	0.65	0.71	0.60	0.69	0.71
H-donor	$h_D^*$ values							
Phenol <sup>a</sup>	0	0	0	0	0	0	0	0
I-Naphthol <sup>b</sup>	0.18	0.06	0.17	0.12	0.21	0.08	0.09	0.06
Carbazole <sup>b</sup>	-1.24	-1.22	-1.53					
Indole <sup>b</sup>	-1.37	-1.40	-1.41		-1.39	-1.42	-1.42	-1.43
Pyrrrole <sup>b</sup>	-1.64	-1.69	-1.60					
Methanol <sup>c</sup>	-1.74	-1.85	-1.20	-1.77	-1.69	-1.72	-1.71	-1.80

<sup>a</sup> Reference 34, <sup>b</sup> Reference 35, <sup>c</sup> Reference 93.

$h_D^*$  values for I-naphthol, methanol, indole, carbazole, and pyrrole. This initial set of  $h_D^*$  values may then be used to determine the  $h_A^*$  values of the series of hydrogen acceptors shown in Table VII in a similar manner to that described previously. The calculated  $h_D^*$  values obtained from the equilibrium constants for the interaction of each hydrogen donor with each base show a reasonable agreement with only one or two exceptions, thus indicating the general applicability of Eq. 36 to hydrogen-bonding systems.

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Address all correspondence to Prof. T. Higuchi, The University of Kansas, Lawrence, KS 66044

## Studies on the Muscarinic and Antimuscarinic Activity of Benzyltrimethylammonium Bromide (BTM)

S. J. STRYCKER\* and J. P. LONG

**Abstract** □ Superfusion of an isolated segment of guinea pig ileum with BTM effectively blocks the contractions induced by histamine, potassium chloride, acetylcholine, and BTM itself for a period of 60–90 min. Spontaneous contractions are also eliminated during this period. Incorporation of atropine in the superfusate removes the blocking activity of BTM, while hexamethonium has no effect on BTM blockade under similar experimental conditions. Perfusion of isolated gut loops of dogs by BTM causes blockade of contractions induced by histamine and acetylcholine. Epinephrine responses were unaffected. However, BTM caused a reduction in the perfusion pressure response to acetylcholine and histamine while the epinephrine response was increased. The pressor response of epinephrine and the depressor response of acetylcholine were significantly reduced in both the systemic and perfused limb blood pressures of the dog following i.m. administration of 1 mg./kg. of BTM.

**Keyphrases** □ Benzyltrimethylammonium bromide (BTM)—activity □ Muscarinic, antimuscarinic activity—BTM □ Ileum-jejunum, isolated—superfusion technique □ Systemic, perfused limb pressures—BTM effect

The cholinergic properties of benzyltrimethylammonium ion (BTM) were first reported by Alles (1) in 1944. A subsequent study by Lee and Shideman (2) in 1959 showed that BTM exhibited both muscarinic and nicotinic activities in certain preparations, with muscar-

inic activity predominating. This finding was substantiated by Hamilton and Rubenstein (3) in 1968. They described the dual muscarinic and nicotinic activities of BTM plus its pyridyl analogs. Publications by Wong and Long (4) in 1962 and by Long, Wong, and Witt (5) in 1965 first revealed that the parent compound plus some of its halogenated isomers exhibited anticholinergic activity at higher dosage levels in addition to their cholinergic response. The studies reported in this paper were carried out in an attempt to evaluate the scope of the anticholinergic response and to investigate the extent to which BTM was capable of antagonizing the effects of other agents.

#### MATERIALS AND METHODS

**Isolated Guinea Pig Ileum**—The *in vitro* anticholinergic activity was evaluated using the superfused guinea pig ileum (6) obtained from more than 50 animals. The guinea pigs were stunned by a blow on the head, and a 20–30-mm. segment of the lower ileum was removed. The force of contraction of the gut segment was measured with a Statham strain gauge and recorded with an Offner-type RS dynograph. Each ileum preparation was subjected to an initial tension of 1 g., and each gram of force of contraction was calibrated to produce 1.0 cm. of displacement on the record. The ileum strips were constantly superfused with warmed (37°) Tyrode's solution aerated with bubbling 95% oxygen–5% carbon dioxide at a rate of 7 ml./min. by means of a Sigmamotor-type T-8 peristaltic infusion