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Thermodynamic parameters of molecular complexes in aqueous solution: enthalpy-entropy compensation in a series of complexes of caffeine with β-naphthoxyacetic acid and drug-related aromatic compounds

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Stability constants and thermodynamic parameters have been evaluated for the complexation reaction in aqueous solution of caffeine with β-naphthoxy acetic acid. The values were higher than those previously reported for the complexation of other ligands with methyl xanthines. In nearly all aromatic ligands complexing with caffeine and theophylline for which data are available, both entropy and free energy of complexation were linearly related to the enthalpy, giving an isoequilibrium relationship. Salicylamide, sodium benzoate and *cis*-methyl cinnamate exhibited slight deviations on the ΔG-ΔH plot; the non-aromatic dehydroacetic acid showed the largest deviation. The isoequilibrium relationship was shown to be valid statistically (349–365 K, caffeine systems; 353–372 K, caffeine and theophylline systems) indicating underlying chemical causation. Thermodynamic equations are presented for analysis of the factors involved, which are attributed to a combination of substrate-ligand interactions and solvent effects. The substrate-ligand overlap area is considered as a common parameter through which the solvent and interaction forces might cooperate to give rise to linearity in the isoequilibrium relationship. The increasingly negative experimental values of the enthalpy and entropy with increase in ligand planar overlap area are discussed in relation to the underlying forces involved in the complexation.

In molecular complexation and association phenomena, free energy reduction may be enthalpy or entropy driven, if not both. Where reaction is due predominantly to hydrophobic forces, the signs of these thermodynamic parameters differ in the two proposed models: a positive entropy change characterizes associations driven by solvent-structuring effects (Kauzmann 1959; Nemethy & Scheraga 1962) whereas according to the solvent cavity theory (Sinanoğlu & Abdulnur 1964), both functions have overall negative values with the enthalpy dominant. Xanthine and purine complexes have been extensively studied but, as yet, no evidence has been presented of any systematic relation between the free energy and either of the other two parameters. With regard to self-association of purine and pyrimidine derivatives in aqueous media, negative values of ΔH and ΔS uncorrelated to ΔG have been reported (Ts'O et al 1969).

Only for complexes of caffeine are thermodynamic parameters available for a series of ligands interacting with the same substrate. Again, ΔH and ΔS values are mostly negative and tend to increase with the free energy change (Donbrow et al 1976). Aspirin exceptionally was reported to show a slight positive entropy change (+0·6 e.u.) (Higuchi & Zuck 1953). The ligand structures for which these data have been reported are in the main benzoic acid derivatives and perhaps as a consequence, the values are rather limited in range with $-\Delta H$ mostly falling between 3 and 6 kcal (12·5–25 kJ) mol^{-1} and $-\Delta S$ between 4 and 10 e.u. Butyl paraben has the largest recorded values: $-\Delta H$, 7 kcal (29 kJ) mol^{-1} and $-\Delta S$ 15 e.u.

For evaluating relationships between the magnitudes of the thermodynamic parameters, a caffeine complexing system was sought which would extend the range of the data well beyond the previous limits by alteration of the ligand structure. β-Naphthoxyacetic acid (β-NAA) was selected because of its reportedly high $K_{1:1}$ values (Donbrow & Jan 1967). Furthermore, this compound would

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provide the first thermodynamic data on a naphthalene ligand for comparison with benzenoid ligands.

Previous work implicated the possibility of a donor-acceptor mechanism with caffeine as the acceptor (Donbrow & Jan 1967), which could account for enhanced complexation in naphthalene derivatives and other molecules of increased donor capacity. In addition, there seems to be a relationship between the stability constants of a series of theophylline complexes in water and the maximal overlap area of the interactants (Cohen & Connors 1970). In this respect, complexation between deoxyguanosine and the large planar ring actinomycin molecule gives rise to values much larger than those reported for any caffeine-benzenoid ligand systems: $-\Delta H$ 10·3 kcal (42 kJ) mol^{-1} and $-\Delta S$ 19·8 e.u. (Crothers & Ratner 1968).

We have compared these latter values with those of the caffeine β-NAA complex, since both involve enlarged conjugated ring systems capable of increasing the maximum planar overlap area.

MATERIALS AND METHODS

β-NAA and caffeine were recrystallized and both had the correct literature mp. and u.v. spectra.

The method for the determination of stability constants was that of Higuchi & Zuck (1953). The system was shaken for a minimum of 30 h, aliquot portions were filtered at the reaction temperature and the filtrates analysed.

The analytical procedure used was spectrophotometric analysis at 325 μm, at which wavelength the contribution of caffeine is negligible (ratio 1 to 350). The temperature range was extended to 45 °C.

Stability constants (K) representing 1:1 complex formation were calculated from the slope of the initial linear portion of the phase diagrams (Fig. 1) using:

$$K = \frac{\text{Slope}}{(1 - \text{Slope})S_0} \quad (1)$$

where S_0 = saturation solubility of the acid in water.

The enthalpy, ΔH, was obtained from the slope of a Van't Hoff plot of log K against 1/T.

The free energy of binding, ΔG, and the entropy of the reaction, ΔS, were calculated from the relationships:

$$\Delta G = -RT\ln K \quad (2)$$

$$\Delta S = \frac{\Delta H - \Delta G}{T} \quad (3)$$

RESULTS

The phase diagrams at 25 °, 35 °, and 45 °C are shown in Fig. 1. They are similar to those of the naphthalene acid derivatives studied and have been discussed by Donbrow & Jan (1967).

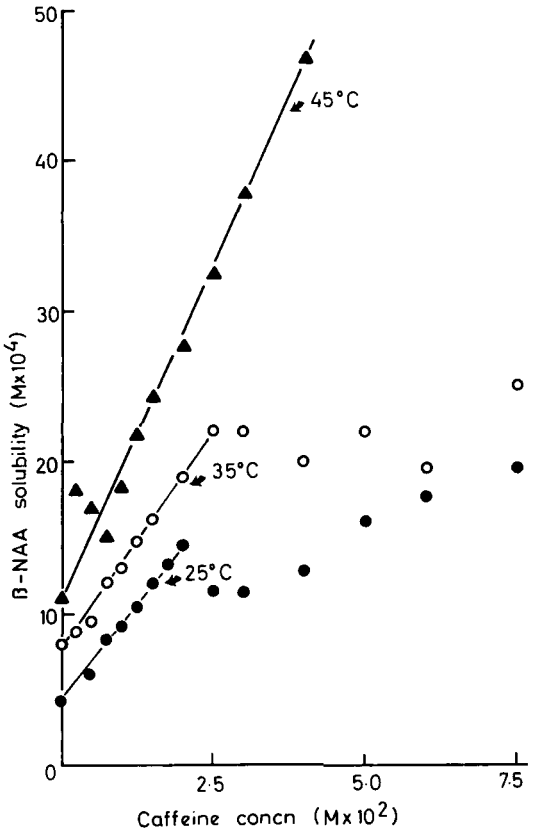

FIG. 1. Phase diagram for β-naphthoxyacetic acid-caffeine complexation in aqueous solution.

Apparent $K_{1:1}$ values are listed in Table 1 together with acid solubilities and percent solubility increases in caffeine solution at maximum solubility which show that very high solubility increases may be achieved. The Van't Hoff plot was linear between 15 ° and 35 °C over which range the enthalpy was independent of temperature (Fig. 2). Such linearity has been proposed as a test for the presence of a single 1:1 complex or isomeric complexes (Orgel & Mulliken 1957; Kuntz & Johnston 1967). At 45 °C, $-\Delta G$ was the same as at 25 °C and the percentage solubility increase rose. These anomalies may indicate a change in stoichiometry. The calculated thermodynamic constants are included in Table 2.

Table 1. Stability constants and solubilities for β-NAA – caffeine systems at different temperatures.

Temp. °C	β-NAA S_0 10^4M[a]	Slope × 10^2	Stability constant $K_{1:1}$ (M^{-1})	% Solubility increase[b]
15	2·5[c]	5·6[c]	239[c]	432
25	4·33	5·1	129·1	237
35	8·1	5·4	70·5	167
45	11·0	9·5	94·5	—

a. Saturation values in water.
b. % increase in β-NAA solubility at precipitation of the complex.
c. Values from Donbrow & Jan (1967) or calculated from their data by present authors.

DISCUSSION

As was expected on the basis of the enlarged maximum overlap area, or alternatively the increased interaction energy between molecules, provided by the presence of the second ring, the free energy change for β-NAA-caffeine complexation was much higher than the values previously reported for other caffeine-ligand systems. In parallel with this, the enthalpy and entropy changes were significantly increased. Table 2 also lists reported thermodynamic constants for other caffeine-ligand systems together with values for salicylic and dehydroacetic acid calculated by us using published stability constants (Goto et al 1968) which gave linear van't Hoff plots.

The negative enthalpy for β-NAA and all other caffeine-ligand systems indicates that the reaction is exothermic while the negative entropy suggests a higher degree of ordering in the system in the complexed state. The β-NAA values lie close to those of the actinomycin-deoxyguanosine system and although the three condensed rings of actinomycin are larger in area, the maximum planar overlap area is clearly determined by deoxyguanosine, the area of the guanine portion being similar to that of caffeine.

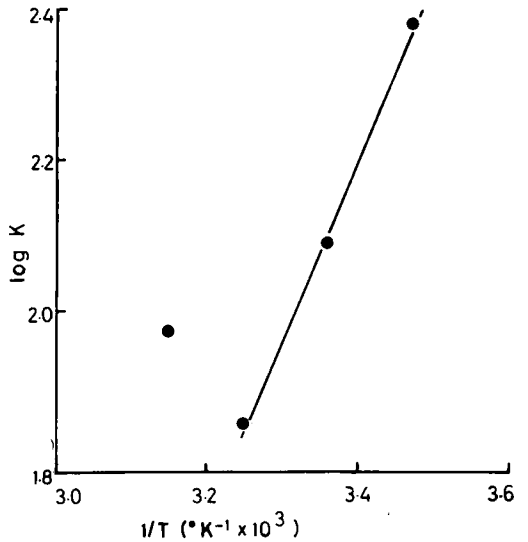

FIG. 2. van't Hoff plot for β-naphthoxyacetic acid-caffeine complex.

Table 2. Thermodynamic parameters for caffeine-ligand systems.

Ligand	Temp. °C	$-\Delta G^7$ k cal mol^{-1}	$-\Delta H$ k cal mol^{-1}	$-\Delta S$ cal mol^{-1} deg	Ref.[8]
Aspirin[1b,2]	15	1·71	1·5	−0·6	(a)
	30	1·72			
Benzoic acid [1a]	0	1·83	3·0	4·0	(a)
	15	1·80			
	30	1·75			
Sodium benzoate[1c]	0	1·70	3·3	6·0	(a)
	15	1·60			
	30	1·54			
Salicylamide[1b,3]	15	2·45	3·9	5·0	(b)
	25	2·40			
	(30)	(2·38)			
	37	2·38			
Tryptophan[1b,5]	(15)	(2·08)	4·0	6·7	(c)
	25	2·01			
	(30)	(1·98)			
Salicylic acid[1b,5]	(15)	(2·42)	5·3	10·0	(d)
	30	2·28			
	33	2·25			
	37	2·21			
Riboflavine[1d,6]	8	2·31	5·7	12·0	(e)
	(15)	(2·28)			
	25	2·13			
	30	2·14			
	35	2·04			
Butyl paraben[1b]	15	2·58	7·0	15·0	(a)
	30	2·37			
β-Naphthoxy acetic acid[1b]	15	3·14	10·1	24·1	(f)
	25	2·90			
	35	2·59			
	45	2·90			
Dehydroacetic acid[1b,d,5]	(15)	(1·58)	5·4	13·3	(d)
	30	1·38			
	37	1·22			
	45	1·18			
	60	0·97			

Key
1. Methods. (a) Partition, (b) Solubility, (c) Reverse solubility, (d) Kinetic, (e) Optical rotatory dispersion.
2. Goto et al (1968) used a kinetic method over the range 30–60 °C. Their data are not included here due to scatter in the van't Hoff plot.
3. K measured at 15, 16, 25, 37, 45 °C.
4. K measured at 15, 25, 40, 55 °C.
5. Based on our van't Hoff plot of the published stability constants at 30, 37, 45 and 60 °C. Ethyl *p*-hydroxybenzoate data from this reference did not give a linear plot.
6. K measured at 8, 25, 30, 35, 50, 70 °C. Other methods cited include spectral and solubility.
7. Bracketed values calculated from ΔH and ΔS.
8. Refs. (a) Higuchi & Zuck (1953), (b) Donbrow et al (1976), (c) Nakano & Higuchi (1968), (d) Goto et al (1968), (e) Guttman (1962), (f) Present work and Donbrow & Jan (1967).

Literature data have been omitted from Table 2 for caffeine-ligand systems in which thermodynamic constants were obtained by calorimetric methods at one temperature only (25 °C). Thus sodium salicylate was reported to have high ΔH and ΔS values, ($-\Delta H$ 8·65, 7·0 kcal (36, 29 kJ) mol^{-1} and $-\Delta S$ 24·5, 18·3 e.u.) but relatively low $-\Delta G$ values (1·37, 1·54 kcal (5·7, 6·4 kJ) mol^{-1} at 25 °C) (data from Stern

1974; Rohdewald & Baumeister 1969, respectively) The latter also report values for other benzoates with caffeine which show the same low $-\Delta G$ phenomenon. Urea-caffeine complexation has a low enthalpy (−3·6 kcal (−15 kJ) mol^{-1}), determined calorimetrically at 25 °C (Cesaro et al 1976). On the other hand, ethidinium bromide, a molecule interesting in view of its tricyclic phenanthrene-related structure, forms a strong complex with caffeine, the comparatively high $-\Delta G$ value (2·87 kcal (12 kJ) mol^{-1} at 25 °C) according, as in β-NAA, with the potentially high overlap area, yet the $-\Delta H$ and $-\Delta S$ values were much lower (−5.4 kcal (−23 kJ) mol^{-1}, 8·5 e.u.) than for β-NAA-caffeine (Cesaro et al 1976.)

Whilst considering these structure-thermodynamic parameter relationships it is also worth including data reported for association of caffeine in aqueous solution. Using Higuchi & Guttman's (1957) dimerization constants based on the partition method, we obtained the values: $-\Delta G$ 1·78, 1·57 and 1·48 kcal (7·5, 6·6, 6·2 kJ) mol^{-1} at 0 °, 20 ° and 30 °C, respectively, $-\Delta H$ 4·4 kcal (18·4 kJ) mol^{-1} (linear Van't Hoff plot), $-\Delta S$ 9·6 e.u. Gill et al (1967), using two methods of analysis of calorimetric data for multiple association at 25 °C, obtained lower $-\Delta H$ values, 3·4 and 2·6 kcal (14·2, 10·9 kJ) mol^{-1}. On the basis of the larger $-\Delta H$ value, $-\Delta S$ was 6 e.u. while $-\Delta G$ was 1·5 kcal (6·3 kJ) mol^{-1}. Cesaro et al (1976) obtained $-\Delta S$ 7 e.u. from their calorimetric data at 25 °C. The $-\Delta G$ values are low in relation to the $-\Delta H$ values, compared with the data for caffeine complexes in Table 2.

Few thermodynamic constants have been measured for complexes with theophylline as ligand. Of interest are the data of Connors et al (1969) on complexation with *cis*- and *trans*-isomers of methyl cinnamate, the *trans* giving the higher constant ($-\Delta G$ at 15 °C; *trans*- 1·99 (8·3), *cis*- 1·35 (5·6) kcal (kJ) mol^{-1}; $-\Delta H$, $-\Delta S$; *trans*- 2·9 (12·1), 3·0, *cis*- 1·9 (7·9), 1·9, kcal (kJ) mol^{-1}, e.u. respectively). Probably, plane to plane superimposition is optimized in the *trans*- isomer, affording greater energetic interaction, perhaps as a result also of the higher polarizability of this isomer, whereas in the *cis*-compound, overlapping is hindered by the out-of-plane ester group. The complex of *trans*- methyl cinnamate with 8-chlorotheophyllinate anion (Connors & Sun 1971) possesses higher $-\Delta H$ and $-\Delta S$ values (3·3 kcal (13·8 kJ) mol^{-1}, 5 e.u.) than the theophylline complexes whereas $-\Delta G$ is lower (1·86, 1·80, 1·75 kcal (7·8, 7·5, 7·3 kJ) mol^{-1} at 15, 25 and 30 °C), the seemingly low $-\Delta G$ with high $-\Delta H$ values repeating the phenomenon previously noted with calorimetric data for benzoate anion-caffeine complexes, and also the benzoate data in Table 2, but opposite to that occurring with the aromatic cation ethidinium bromide. Finally, complexation of tryptophan was studied with both caffeine and theophylline, the latter giving fractionally higher values of the constants (Nakano & Higuchi 1968).

Relationship between ΔG, ΔH and ΔS

In all the xanthine systems, the association is enthalpy driven, with entropy as a destabilizing factor. The relative importance of these opposing influences was examined by plotting ΔH against ΔS (Fig. 3) and ΔG (Fig. 4) at 15 ° and 30 °C for a range of ligands. Where one of these temperatures had not

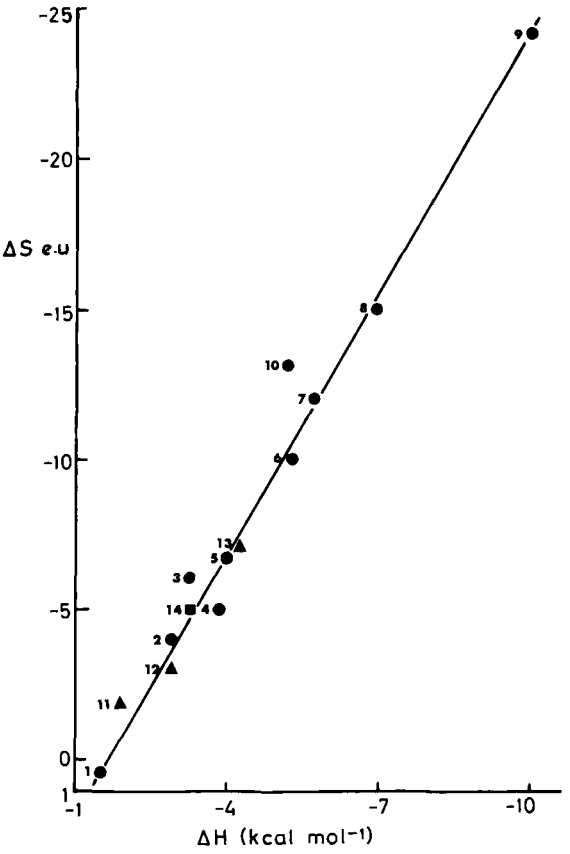

FIG. 3. Relationship of enthalpy and entropy in methylxanthine complexes. Caffeine complexes (●): 1 Aspirin. 2 Benzoic acid. 3 Sodium benzoate. 4 Salicylamide. 5 Tryptophan. 6 Salicylic acid. 7 Riboflavine. 8 Butyl paraben. 9 β-Naphthoxyacetic acid. 10 Dehydroacetic acid. Theophylline complexes (▲): 11 *cis*-Methyl cinnamate. 12 *trans*-Methyl cinnamate. 13 Tryptophan. 8-Chlorotheophylline complex (■): 14 *trans*-Methyl cinnamate.

been used in the measurements, ΔG was calculated using equation 3 with the reported ΔH and ΔS constants. Calorimetric data measured at a single temperature were not included. Theophylline data have been added for comparison with caffeine complexes.

From Fig. 3 it appears that ΔH and ΔS are linearly related for caffeine complexes with β-NAA, butyl paraben, riboflavine, salicylic acid, trytophan, benzoic acid and aspirin. In contrast, the points for benzoate ion, salicylamide and dehydroacetic acid are scattered. It is tempting to rationalize this linearity on the basis of possession of common structural features, in particular an aromatic ring substituted with an unsaturated group (C=O or C=C) or unsaturated fused ring. The absence of the aromatic nucleus (dehydroacetic acid) or the presence of certain other structural features such as an *o*-hydroxy amide substituent (salicylamide) or an ionic group (benzoate) may be the cause of deviations from the relation, these features being known to modify substantially donor-acceptor properties or water structuring (the anionic 8-chlorotheophyllinate substrate might also be expected to cause water structuring. This molecule however has a high capacity for charge delocalization.)

The plot of ΔG against ΔH (Fig. 4) points to a similar structural differentiation but with greater scatter. The deviations of the benzoate* or, more strikingly, the non-aromatic substrate are opposite to that of salicylamide in both figures and the causes could be enthalpic or entropic. The data on complexes of theophylline and its 8-chloro anionic derivative with *trans*-methyl cinnamate fall reasonably close to the caffeine-ligand line whereas the *cis*-isomer deviates (Figs 3, 4).

Since the relationship encompasses K values covering two orders of magnitude with a tenfold range in ΔH and twenty five-fold in ΔS, the validity would not appear to be questionable. The result is remarkable for another reason: the K values were measured by a variety of methods (See Table 2, footnote 1) and over different concentration ranges, yet the linearity accommodates values uncorrected (except in benzoic acid) for the possible presence of complexes higher than 1:1 or caffeine dimerization. This could imply additivity in the compensation relationships involving these factors or that the corrections are negligibly small. ΔG is in fact less

* The calorimetric data for the other caffeine-substituted benzoate systems (loc. cit.) show a similar deviation, whereas ethidinium bromide is displaced to the opposite side.

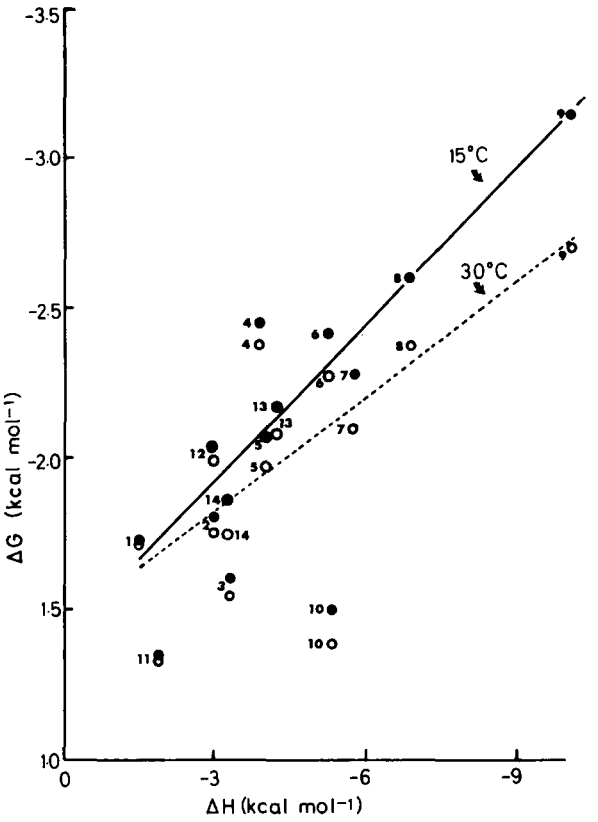

FIG. 4. Relationship of free energy and enthalpy in methylxanthine complexes. For key to compounds see Fig. 3 legend.

sensitive to deviations than K. Nevertheless, apart from the grossly deviant points referred to previously, the systems lie within about ±5% of the line (Fig. 4), and the corresponding error range of ±20% in K may be wide enough to include variations due to the use of uncorrected K values. The possibility of a systematic deviation proportional to the value of K arising from the presence of higher complexes cannot be excluded, but this would not alter the conclusions, though changing the slope of the line.

The correlation between ΔH and ΔS would imply that they are inter-connected through an isoequilibrium relationship (Leffler 1955) of the compensation type characterized by having an isoequilibrium temperature (β) greater than the experimental temperature (T) used and with the selectivity decreasing (Exner 1972). Compensation relationships have been reported for iodine complexation with amines in non-aqueous solvents and other charge-transfer complexes (Brieglieb 1961; Foster 1973) but not for xanthines or for complexation in aqueous systems.

A genuine isoequilibrium relationship is thought to exist where there is a large difference between T and β, a significant range in both ΔH and ΔS and a linear ΔG-ΔH relationship. All these criteria are observed by the present system. Nevertheless, some doubt has been expressed in the literature about the validity of such relationships in a number of reactions in which the apparent linearity is the result of statistical compensation (Leffler 1955; Ritchie & Sager 1964; Exner 1972).

Several tests have been proposed for estimating the validity of an underlying chemical relationship between ΔH and ΔS (Exner 1972). Krug et al (1976) have recently proposed the calculation of 95% confidence limits (CI) of the slope, β, of the regression of ΔH on ΔS as a hypothesis test to discriminate between a random statistical compensation pattern and the linear chemical compensation pattern.

$$\Delta H = \beta\Delta S + \Delta G \text{ (at } T = \beta) \qquad (4a)$$

or

$$\Delta H = \beta\Delta S + \Delta G_\beta \qquad (4b)$$

Since the two linear compensation patterns are identical except for one parameter, it is sufficient merely to test the value of that parameter. The null hypothesis is then $H_o: \beta = T_{hm}$, where T_{hm} is the harmonic mean temperature. Unless this hypothesis can be rejected, there is no reason to suspect the existence of chemical causation. The 95% confidence interval (CI) of β calculated for the regression line of ΔH on ΔS using the Table 2 data (omitting dehydroacetic acid) is 318–380 K (correlation coefficient r = 0·995). However, the mean harmonic temperature, T_{hm} of the nine systems is ca 296 K (range 288–305 K)* which lies well outside the confidence limits, hence the null hypothesis must be rejected and it may be concluded that the linear compensation is not statistically determined. Inclusion of the four theophylline systems in the calculation gives 328–379 K for CI 95% (r = 0·994), with T_{hm}297 K, indicating that their addition does not change the above conclusion. There is therefore reason to look for the existence of a structure-dependent relationship between ΔH and ΔS in this series of xanthine complexes. (Krug et al (1976) were unable to discover any such genuine linear compensations for equilibrium reactions in the literature.)

It was recently suggested that the best estimates of the parameters and the intercept are obtained from ΔH regressions on ΔG at T_{hm} rather than ΔH on ΔS, because of the large covariance between estimates of the latter parameters, statistical independence existing only in the former plane (Krug et al 1976). By combination of equation (5) for the ΔH-ΔG regression (slope γ, intercept ΔH_β) with equations (3) and (4b), the slopes and intercepts are evaluated via equations (6a) and (6b):

$$\Delta H = \gamma\Delta G + \Delta H_\beta \qquad (5)$$

$$\beta = T/(1 - 1/\gamma) \qquad (6a)$$

$$\Delta G_\beta/\beta = \Delta H_\beta/\gamma T \qquad (6b)$$

$-\Delta G_\beta/\beta$ is the intercept of ΔS on ΔH (cf. Fig. 3) and $T\Delta G_\beta/\beta$ of ΔG on ΔH (cf. Fig. 4).

Values for the parameters and the 95% CI calculated using the four possible regressions for the 9 caffeine and the 13 caffeine + theophylline systems are listed in Table 3. Differences between the first three are unsubstantial, possibly due to statistical interdependencies, and the values agree closely with the lines in Figs 3 and 4, which were drawn weighted according to data value distribution, slightly favouring higher values. However, the fourth method (ΔH on ΔG) gave β values some 4% higher and intercepts 13% lower, reflecting the greater number of low value data points in the region of substantial scatter.

Table 3. Statistically evaluated slopes and intercepts, with 95% confidence interval (CI).

Regression[1]	n	β K	CI (95%)	T_{hm} K	$-\Delta G_\beta/\beta^2$	r
ΔH on ΔS	9	349	318–380	296	4·8	0·99
	13	353	328–379	297	4·6	0·99
ΔG on ΔH	9	349	322–382	296	4·8	0·91
	13	354	331–381	297	4·5	0·91
ΔS on ΔH	9	353	324–387	296	4·7	0·99
	13	357	334–385	297	4·5	0·99
ΔH on ΔG	9	365	339–443	296	4·2	0·91
	13	372	348–428	297	3·9	0·91

1. Experimental ΔG values at 288 were used or were calculated from K values, where available. n is the number of data points included (9 for caffeine and 4 for theophylline systems).
2. Intercept of ΔS on ΔH (cf. Fig. 3) in calories calculated from statistically estimated intercept using eqn (6b) or (4b). Intercept of ΔG on ΔH (Fig. 4) is given by $T\Delta G_\beta/\beta$, where TK is the experimental temperature.

For the caffeine complexes the parameters range from 349 to 365 K for β and 4·8 to 4·2 for $\Delta G_\beta/\beta$. The theophylline complexes appear to conform with the caffeine pattern; however, they do exhibit deviations, possibly due to the rather varied structures involved. There are too few compounds to enable a

* Although experimental temperatures are not identical in all systems, they lie within a fairly close range. Van't Hoff's equation was valid and gives the best possible ΔH estimates. T_{hm} was calculated for each system, omitting higher temperatures (> 40 °C) where three or more data points were available in the lower range.

firm conclusion to be drawn at this time. The large positive intercept value is surprising, the origin having been used in many systems as a definitive point in measuring β values (Leffler & Grunwald 1963). It contributes, at zero enthalpy change, as much as −1·38 kcal (5·77 kJ) mol^{-1} to $-\Delta G$ at 15 °C, derived apparently from an underlying positive entropy change, which is discussed later.

Since the K value data sources are in cm^3 mol^{-1} units, they include the cratic entropy loss of some 8 e.u. entailed in the combination of two molecules in the complex (Gurney 1953). The unitary (u) parameters are given by:

$$\Delta S_u^0 = \Delta H\beta + 12{\cdot}8 \text{ (to } 12{\cdot}2\text{) e.u.} \quad (4c)$$

$$\Delta G_u^0 = (1 - T/\beta)\Delta H - 12{\cdot}8T \text{ (to } 12{\cdot}2T\text{) cal} \quad (7)$$

Clearly, the positive unitary entropy change at zero ΔH is quite substantial (TΔS 3·7 kcal (15·5 kJ) at 15 °C). Such intercepts could be artifacts due to scatter or curvature at low $-\Delta H$ values, but there is evidence for their reality in some systems (Brieglieb 1961) and they may be attributable to effects of the solvent on a common substrate (see last section).

In these xanthine complexing systems there is reasonable sensitivity in the ΔG − ΔH plots at 15 °C and 30 °C as full compensation of ΔH and TΔS, at which ΔG is invariant for all the complexes, is not reached till about 78 °C (T= β, eqn 7). Nevertheless, the destabilizing influence of TΔS in this type of compensation is responsible for the much smaller relative increase in $-\Delta G$ between the weakest and strongest complexes than in $-\Delta H$.

Extrathermodynamic relationships and compensation effects

From the work of Cohen & Connors (1970) and Connors et al (1969) on complexation of xanthine derivatives with aromatic ligands in water, there would appear to be an extrathermodynamic relationship between the free energy of complexation and the interactant area or maximum overlap area (MOA) of substrate and ligand based upon projections from molecular models. Such a relationship could imply the existence of extra-thermodynamic relationships between MOA and both ΔH and ΔS of complexation for a given series (unless one of these parameters were constant or negligible). The compensation between ΔH and ΔS found in the present work suggest that ΔH, the apparent source of the negative free energy change, should be linearly related to the MOA.

For analysis of the effects contributing to ΔH and ΔS, a simple additivity principle may be used as a first approximation. Assuming partial processes to be involved, ΔG_u^0 the unitary free energy change for complex formation may be divided into ΔG_{AB}^0 and ΔG_S^0, the free energy changes for the direct substrate-ligand interaction and the solvent effects, respectively, to give,

$$\Delta G_u^0 = \Delta G_{AB}^0 + \Delta G_S^0 \quad (8)$$

ΔG_{AB}^0 may involve donor-acceptor, charge-transfer, dipole-dipole, dipole-induced dipole and/or van der Waals interaction forces, which in the first approximation are assumed to be all functions of the chemical parameters reponsible for the extrathermodynamic relation. ΔG_S^0 contains all solvent effects involving energy differences between the complex and the separate substrate and ligand molecules, including hydrophobic bonding and solvation. The reason why a chemical as well as a solvent-free energy term is included is because (i) the β value found here is above the range (280–320 K) considered indicative of reactions driven only by water as solvent (Lumry & Rajender 1970); (ii) physicochemical changes in u.v. spectra and o.r.d. used in the analysis of certain of the systems may arise from solute-solute interactions; (iii) although change of solvent from water to organic solvent-water mixtures reduces complexation strength greatly, the solvent effect does not account for the whole of the free energy change (Connors & Sun 1971).

Dividing free energies into corresponding enthalpies and entropies

$$\Delta G_u^0 = \Delta H_{AB}^0 - T\Delta S_{AB}^0 + \Delta H_S^0 - T\Delta S_S^0 \quad (9)$$

Each of the terms may be composite. The solvent effects could include cavity formation (c), water-structuring (str) and differential solvation (ds), full expansion giving:

$$\Delta G_u^0 = \Delta H_{AB}^0 - T\Delta S_{AB}^0 + \Delta H_c^0 - T\Delta S_c^0 + \Delta H_{str}^0 - T\Delta S_{str}^0 + \Delta H_{ds}^0 - T\Delta S_{ds}^0 \quad (10)$$

The substrate-ligand terms may be subdivided similarly. Again additivity is assumed and parameter interaction neglected. For entropy-enthalpy compensation to occur, independent quasi-isoequilibrium relationships should hold for each effect involved in the reaction, taking the form:

$$\delta_i\Delta H_i^0 = \beta_i\delta_i\Delta S_i^0 \quad (11)$$

$$\text{where } \delta_i\Delta H_i^0 = \left(\frac{\partial \Delta H^0}{\partial r_i}\right)_{R_0,T} dr_i$$

$$\text{and } \delta_i\Delta S_i^0 = \left(\frac{\partial S^0}{\partial r_i}\right)_{R_0,T} dr_i$$

dr_i represents a temperature-independent variable associated with the specific interaction effect (i) on

changing from a given ligand structure (R_0 the reference compound) to another (R), following Leffler & Grunwald's notation (1963) and β_i the proportionality constant. Linear combination of terms for each possible mechanism yields an equation for the overall effect of ligand structure change, δ_R, on the enthalpy-entropy relation:

$$\delta_R\Delta H^0 = \delta_1\Delta H^0_{AB} + \delta_2\Delta H^0_C + \delta_3\Delta H^0_{str} + \delta_4\Delta H^0_{ds} = \beta_1\delta_1\Delta S^0_{AB} + \beta_2\delta_2\Delta S^0_C + \beta_3\delta_3\Delta S^0_{str} + \beta_4\delta_4\Delta S^0_{ds} \quad (12)$$

Similar equations may be developed for the relation of free energy to entropy. They show that for multiple interaction mechanisms, observed values of ΔG^0, ΔH^0 and ΔS^0 are the sum of two or more independent vectors. This should normally lead to scatter in the isoequilibrium plots (Leffler & Grunwald 1963).

The existence of a good experimental isoequilibrium relationship implies either dominance of one mechanism over the others or the presence of a constant factor determining the value of the proportionality constant over the whole series of structures such that

$$\beta_1 = \emptyset_1\beta_2;\ \beta_2 = \emptyset_2\beta_3 \text{ etc.} \quad (13a)^*$$

$$\Delta S^0_C = \Psi_1\Delta S^0_{str};\Delta S^0_{str} = \Psi_2\Delta S^0_{ds} \text{ etc.} \quad (13b)$$

where $\emptyset_1$, $\emptyset_2$ etc. and Ψ_1, Ψ_2 are constants.

In the present case, such a parameter interaction factor, if present, might well be related to the molecular overlap area of the substrate and ligand in the complexes. Thus the A-B interaction energy for a series of ligands and a common substrate involves parameters related to the dimensions of the ligand conjugated system where II-electron, orbital overlap or polarization mechanisms are implicated†. The extent of the conjugated region in the ligand (generally the aromatic ring with its immediate substituent groups) also probably determines the overlap area in a sandwich-type complex, provided the substrate planar area (or extension) is the larger.

The planar overlap area determines the free energy of the solvent cavity effect through its influence on the work of surface area change in forming a single cavity for AB from separate A & B cavities and on the associated interfacial entropy change† (Sinanoğlu 1968). Again, the same factor is involved in water-structuring, determining the number of molecules of water liberated into the bulk on complexation and hence the gain in entropy and the associated enthalpy change. The correlation of maximum overlap area with overall free energy change demonstrated by Cohen & Connors (1970) is in accord with these arguments though, because of the compensation effect, the sensitivity of such a correlation is lower than that expected with ΔH or ΔS.

Unfortunately, the substrates in Table 2 are not ideally suited for a critical test of the influence of maximum overlap area on ΔH and ΔS because of the varying steric, conformational and ionic effects present, in some cases associated with the presence of bulky non-planar hydrophobic or hydrophilic substituent groups. Moreover, maximum overlap does not allow for specific orientation imposed upon the molecules by the substantial dipole attraction and repulsion forces present. Nevertheless, measurements were made of the *probable* overlap area using a technique similar to that employed by Cohen & Connors for *maximum* overlap areas and based here on Courtauld Atomic Models. The main differences were that in cases where alternative conformations were possible, these were taken into account and, furthermore, very unlikely overlap configurations involving steric hindrance or electrostatic repulsion were excluded as far as possible. This resulted in a rather wide range of values in the cases of β-NAA and butyl paraben. There does seem to be a correlation between ΔH and the probable overlap area for most complexing systems (Fig. 5). (In riboflavine, tryptophan and aspirin, which have been omitted, large overlap areas were possible that could have been reduced greatly as a result of specific

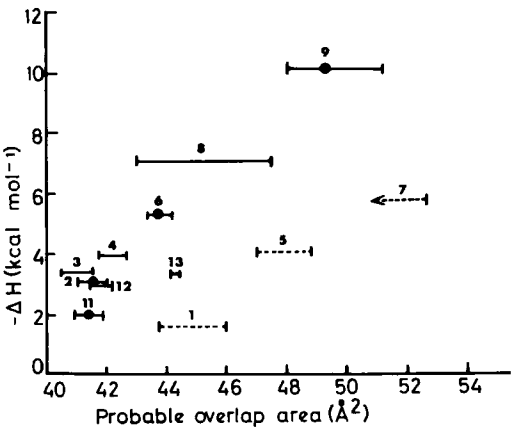

FIG. 5. Relationship of enthalpy to estimated substrate-ligand overlap area in methylxanthine complexes. For key to compounds see Fig. 3. legend.

* The trivial case of accidentally coincident β_1 and β_2 values is not considered.

† The appropriate equations involve molecular area and/or volume parameters. Extrathermodynamic correlations frequently occur with both molecular area and volume (see for example Leo et al 1976).

repulsion or steric effects.) Overlap area correlations are also exhibited by ΔG and ΔS.

The three thermodynamic functions are also related linearly to van der Waals volumes or areas, calculated from Bondi increments, for ligand aromatic rings together with the groups contiguous or conjugated to the aromatic ring. (Discrete groups included COOH, $CONH_2$, $COOCH_2$, $C(CH_2)$=CH but not distal groups, e.g. the second and subsequent groups in an alkyl chain.)

The correlation may be expressed by writing each of the terms in equation (9) as the product of an area term, A, and an intensity term (represented by primes for the strength of the effect per unit area) giving:

$$\Delta G_u^0 = A_1 [\Delta H'_{AB} - T\Delta S'_{AB}] + A_2 [\Delta H'_S - T\Delta S'_S] \quad (14)$$

where $A_1 \approx A_2$.

The progressive increases in $-\Delta H$ and also in $-\Delta G$ and $-\Delta S$ with increasing planar molecular area in the xanthine complexes accords with the maximum overlap hypothesis and frontier orbital theories (Fawzi et al 1980). The entropy loss would become increasingly negative with reduction in the degrees of freedom of the interactants; such reduction is expected to be related to the *total area* of the rigid planar regions of both interactants even if these regions are not fully overlapping. Negative enthalpy change would arise from the restoration of three-dimensional hydrogen bonding in the water molecules displaced from the interactant surfaces in the region of overlap; the number of such water molecules would be related to the actual *overlap area* of hydrophobic groups rather than to the planar area alone. Negative enthalpy change would also be contributed by specific donor-acceptor and other intermolecular interactions. Masked positive enthalpy changes could, however, be present, originating from less-favourable overlap configurations forced upon the molecule by steric repulsion forces including those involving shielding and desolvation of groups. Such enthalpy changes could be among the sources of the deviations observed from the plots in Figs 3, 4 and 5.

On the basis of the above analysis, entropy changes are determined by a different value of the area term than enthalpy changes and solvent effects by a different area value than chemical effects. Such area differences could, however, be insignificant in a closely-related ligand series though acquiring importance in large structures such as riboflavine and molecules in which secondary interactions occur.

It is, however, also necessary to take into account self-compensation of solvent enthalpy and entropy terms. For solvent self-compensation, equation (11) may be written for a specific substrate-ligand interaction in the form:

$$\Delta H_S^0 = \beta_S \Delta S_S^0 \quad (15)$$

where β_S is the compensation temperature parameter. The solvent free energy change during formation of a given complex is

$$\Delta G_S^0 = (1 - T/\beta_S)\Delta H_S^0 \quad (16)$$

The value of β_S is thought to lie between 280 and 320 K for interactions in water (Lumry & Rajender 1970) and when the experimental temperature T lies in the same region as β_S, $\Delta G_S^0 \approx 0$. Consequently $\Delta G_u^0 \approx \Delta G_{AB}^0$ in equation (8), leading to simplification of equation (9) to

$$\Delta G_u^0 \approx \Delta H_{AB}^0 - T\Delta S_{AB}^0 \quad (17)$$

This would imply that the chemical interaction supplies the work-doing part, termed the 'motive' part by Lumry (1980), whereas the enthalpy and entropy changes in the water are irrelevant to the free energy change, and are termed by him the 'fluctuation' part. If the experimental method used measures overall changes in thermodynamic effects, ΔH and ΔS values will also include the changes occurring in the water contained in equation (14), which has the potentiality to explain deviations caused by unusual structural effects and solvent variations through fluctuations in the values of any of its terms. In the caffeine-ligand systems there is conformity to a general compensation relationship and the deviations are small (in all area or volume correlations, the relative positions of salicylamide, salicylic acid and *trans*-methyl cinnamate were higher and that of benzoate lower in ΔG plots than in ΔH and ΔS plots, as in Figs 3 and 4), implying that a common parameter such as an area term seems to govern both chemical and solvent effects.

Signs of the thermodynamic parameters and organic solvent effects

Considering now the signs of the main terms in equation (10), ΔH_{AB}^0 and ΔS_{AB}^0 are expected to be negative for substrate-ligand interactions, the entropy loss resulting from geometrical constraints imposed on the constituents which lead to loss of rotational or vibrational freedom in particular. Connors & Sun (1971) have obtained negative values for the overall ΔH^0 and ΔS^0 in acetonitrile–water mixtures, the values becoming increasingly negative as the water content is reduced, which suggests that

ΔH^0_{AB} and ΔS^0_{AB} remain negative in non-aqueous solvent. ΔH^0_C is the product of interfacial tension and surface area change and is always negative for area reduction. Since ΔH^0_{str} is considered to be close to zero (Kauzmann 1959), the overall ΔH value is negative. The ΔS_c term appears to be negative (Sinanoğlu & Abdulnur 1964) and the ΔS_{str} positive (Kauzmann 1959) as a result of reduction in water-structuring.

The overall negative entropy change observed in this series may mask such an underlying positive entropic contribution to the association. Ts'O et al (1969) have found evidence for such a hidden contribution by comparison of negative entropy and enthalpy values in the stacking self-association of bases (including caffeine) and nucleosides in aqueous solution. An apparent positive entropy contribution was actually demonstrated by Crothers & Ratner (1968) in the actinomycin association with deoxyguanosine: addition of methanol progressively reduced the entropy. Destabilization of association on organic solvent addition results from an increasingly negative entropy of association, which is only partly compensated by an increasingly negative enthalpy of association (see eqn 14). The same changes also occur in the complexation of *trans*-methyl cinnamate and 8-chlorotheophyllinate in the presence of increasing quantities of acetonitrile (Connors & Sun 1971).

It seems likely that the positive entropy intercept noted at zero enthalpy of complexation (eqn 4c) reflects such a positive solvent entropy change for caffeine overlap with a hypothetical aromatic ligand in which the enthalpy change of the chemical interaction is equal and opposite in sign to that of the solvent effect.

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