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Molecular interactions of caffeine with benzoic acid, *o*- and *p*- methoxybenzoic acids and *o*- and *p*-nitrobenzoic acids

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The solubility of the complexes formed by caffeine with benzoic acid and substituted benzoic acids has been studied. An insoluble complex is formed by *o*-methoxybenzoic acid, whereas *p*-methoxy-, *o*- and *p*-nitrobenzoic acids and benzoic acid itself form soluble complexes. The phase diagrams have been analysed to evaluate the approximate stability constants for 1:1 interaction. The stabilities increase as the electron-withdrawing power of the benzoic acid substituent decreases, and there is a correlation between log K values and pK_a values (or σ constants) of the acids. This indicates that the binding strength is related either to the polarity of the carboxyl group or to the electron density of the aromatic ring. The implications of these results are discussed in terms of the mechanism of complex-formation.

A LARGE variety of complexes of caffeine have been examined in an attempt to elucidate the mechanism of complex-formation. Both qualitative and quantitative methods have been employed and stability constants have been reported by Labes (1930) and by Higuchi & Zuck (1952, 1953) and Higuchi & Lack (1954). Of the numerous mechanisms proposed, no single one accounts for the phenomena observed, and in the extensive studies made by Higuchi & others, dipole-dipole interactions, hydrogen bonding and the "squeezing-out" effect of water on the hydrophobic group have been proposed, to explain the constants obtained. Results have been difficult to correlate because of differences in solubility and in the stoichiometric ratios among the complexes, and because mixtures of complexes are formed. Quantitative methods of study have been restricted, since the usual spectroscopic and electrochemical methods are not applicable although the molar ratios may be determined refractometrically (Donbrow & Jan, 1963).

We report initial solubility studies made on a closely-related series of complex-forming molecules.

Experimental

SOLUBILITY STUDIES

An excess quantity of the organic acid was shaken at 15° ± 0·05 with varying weighed quantities of caffeine in water or in mineral acid solution (to suppress ionisation of the organic acid). The time of shaking varied between 8 hr and several days according to the supersaturation tendencies of the system and an intermediate storage period of 12 hr at 5° was interposed.

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An aliquot portion of the solution was removed by suction through a filter stick and its content of organic acid determined either titrimetrically or spectrophotometrically. The method of calculation has been described by Higuchi & Zuck (1953).

TABLE 1. SOLUBILITY OF *o*-METHOXYBENZOIC ACID IN AQUEOUS SOLUTIONS OF CAFFEINE AT 15° AND STABILITY CONSTANTS FOR INTERACTION OF *o*-METHOXYBENZOIC ACID AND CAFFEINE

Caffeine conc. moles × 10^2	Acid conc. moles × 10^2	Complex moles × 10^2	Free caffeine moles × 10^2	K ($moles^{-1}$ litre)
0·000	2·05	—	—	—
0·503	2·27	0·22	0·283	37·92
0·979	2·46	0·41	0·569	35·15
1·497	2·66	0·61	0·887	33·54
1·980	2·87	0·82	1·110	36·03
2·980	3·28	1·23	1·750	34·29
3·48	3·52	1·47	2·010	35·67
3·98	3·70	1·65	2·330	34·50
4·50	3·87	1·82	2·680	33·13
4·98	4·08			
6·02	3·90			
7·98	3·90			
9·03	3·88			
10·06	4·00			
10·99	3·76			
12·03	3·66			
13·50	3·39			
14·83	2·92			

Average K = 34·9 $moles^{-1}$ litre: K value from slope = 34·7 $moles^{-1}$ litre

Quantities of acids used were as follows:

o-*Methoxybenzoic acid* (500 mg) in water (50 ml). Titration indicator, α-naphtholphthalein. The results are listed in Table 1.

p-*Methoxybenzoic acid* (100 and 200 mg) in water (50 ml). Titration indicator as above (Table 2).

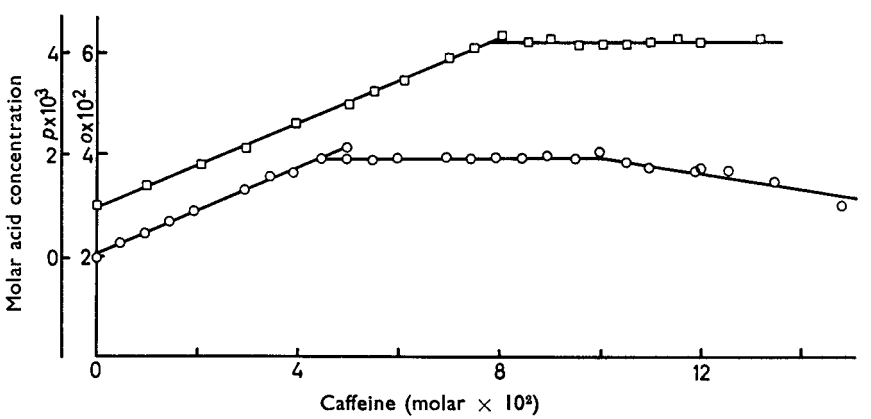

FIG. 1. Solubility of *ortho-* and *para-* methoxybenzoic acids in caffeine solutions. —□— = *para*. —○— = *ortho*.

o-*Nitrobenzoic acid* (500 mg) in 0·005N hydrochloric acid (50 ml). Titration indicator, bromothymol blue (Table 3).

p-*Nitrobenzoic acid* (100 mg) in 0·001N hydrochloric acid (50 ml). Titration indicator as above (Table 4).

Benzoic acid (500 mg) in 50 ml of either water or 0·001N hydrochloric acid. Titration indicator, phenol red (Tables 5 and 6).

TABLE 2. SOLUBILITY OF *p*-METHOXYBENZOIC ACID IN AQUEOUS SOLUTIONS OF CAFFEINE AT 15° AND STABILITY CONSTANTS FOR INTERACTION OF *p*-METHOXYBENZOIC ACID AND CAFFEINE

Caffeine conc. moles × 10^2	Acid conc. moles × 10^3	Complex moles × 10^3	Free caffeine moles × 10^2	K (moles^{-1} litre)
0·00	1·00	—	—	—
0·97	1·40	0·40	0·930	43·0
2·06	1·80	0·80	1·970	40·6
2·95	2·10	1·10	2·840	38·7
3·95	2·60	1·60	3·79	42·2
4·99	2·95	1·95	4·80	40·6
5·57	3·20	2·20	5·35	41·1
6·08	3·40	2·40	5·83	41·1
6·97	3·85	2·85	6·68	42·6
7·50	4·05	3·05	7·20	42·3
8·02	4·30			
9·01	4·25			
10·02	4·10			
11·53	4·20			
13·15	4·20			

Average K = 41·3 moles^{-1} litre: K value from slope = 42·3 moles^{-1} litre

TABLE 3. SOLUBILITY OF *o*-NITROBENZOIC ACID IN CAFFEINE SOLUTIONS CONTAINING 0·005N HYDROCHLORIC ACID AT 15° AND STABILITY CONSTANTS FOR INTERACTION OF *o*-NITROBENZOIC ACID AND CAFFEINE

Caffeine conc. moles × 10^2	Acid conc. moles × 10^2	Complex moles × 10^2	Free caffeine moles × 10^2	K (moles^{-1} litre)
0·00	2·84	—	—	—
0·94	2·93	0·09	0·850	3·73
1·46	3·28	0·44	1·020	15·18
2·20	3·28	0·44	1·764	8·78
3·09	3·65	0·81	2·280	12·50
4·17	3·75	0·91	3·26	9·82
4·98	3·97	1·13	3·85	10·33
6·02	4·15	1·31	4·71	9·79
7·01	4·44	1·60	5·41	10·41
8·01	4·53	1·69	6·32	9·41
8·99	4·78	1·94	7·05	9·69
10·20	5·06	2·22	7·98	9·79
12·00	5·42	2·58	9·42	9·64
13·01	5·38			
14·01	5·40			
15·00	5·48			
16·69	5·48			

Average K = 9·92 moles^{-1} litre: K value from slope = 9·60 moles^{-1} litre

TABLE 4. SOLUBILITY OF *p*-NITROBENZOIC ACID IN CAFFEINE SOLUTIONS CONTAINING 0·001N HYDROCHLORIC ACID AT 15° AND STABILITY CONSTANTS FOR INTERACTION OF *p*-NITROBENZOIC ACID AND CAFFEINE

Caffeine conc. moles × 10^2	Acid conc. moles × 10^3	Complex moles × 10^3	Free caffeine moles × 10^2	K (moles^{-1} litre)
0·00	0·55	—	—	—
1·08	0·70	0·15	1·07	25·6
2·12	0·80	0·25	2·10	21·7
3·17	0·95	0·40	3·13	23·2
3·96	1·05	0·50	3·91	23·2
4·98	1·20	0·65	4·92	24·0
6·08	1·25	0·70	6·01	21·2
7·04	1·35	0·80	6·96	21·0
7·55	1·50			
8·01	1·40			
9·06	1·50			
10·01	1·45			

Average K = 22·8 moles^{-1} litre: K value from slope = 23·0 moles^{-1} litre

TABLE 5. SOLUBILITY OF BENZOIC ACID IN CAFFEINE SOLUTIONS CONTAINING 0·001N HYDROCHLORIC ACID AT 15° AND STABILITY CONSTANTS FOR INTERACTION OF BENZOIC ACID AND CAFFEINE IN 0·001N HYDROCHLORIC ACID

Caffeine conc. moles × 10^2	Acid conc. moles × 10^2	Complex moles × 10^2	Free caffeine moles × 10^2	K (moles^{-1} litre)
0·00	2·06	—	—	—
1·02	2·52	0·46	0·561	39·8
2·66	3·09	1·03	1·630	30·6
4·01	3·63	1·57	2·436	31·2
4·55	3·78	1·72	2·830	29·5
6·01	4·41	2·35	3·660	31·1
7·46	4·78			
8·50	4·80			
9·03	4·91			
10·32	4·80			
11·09	4·89			

Average K = 30·6 moles^{-1} litre (first value disregarded). K value from slope = 30·4 moles^{-1} litre

TABLE 6. SOLUBILITY OF BENZOIC ACID IN AQUEOUS SOLUTIONS OF CAFFEINE AND STABILITY CONSTANTS FOR INTERACTION OF BENZOIC ACID AND CAFFEINE IN WATER

Caffeine conc. moles × 10^2	Acid conc. moles × 10^2	Complex moles × 10^2	Free caffeine moles × 10^2	K (moles^{-1} litre)
0·00	2·05	—	—	—
0·50	2·23	0·18	0·320	27·4
0·97	2·42	0·37	0·604	29·8
1·97	2·80	0·75	1·220	30·0
2·93	3·12	1·07	1·860	28·1
3·48	3·29	1·24	2·240	27·0
3·99	3·51	1·46	2·530	28·1
5·01	3·79	1·74	3·270	26·0
5·96	4·31	2·26	3·700	29·7
6·54	4·49	2·44	4·098	29·0
7·48	4·73	2·68	4·800	27·2
8·96	5·04			
10·02	4·94			
11·02	4·82			
12·01	4·83			
16·33	4·79			

Average K = 28·2 moles^{-1} litre: K value from slope = 27·4 moles^{-1} litre

Discussion

PHASE DIAGRAMS

o-Methoxybenzoic acid. The solubility diagram is shown in Fig. 1. The initial rise is due to formation of a soluble complex and the plateau to the precipitation of a complex. During the latter process the composition of the solution is invariant until the solid acid is exhausted. The length of the plateau is determined by the quantity of excess solid acid in the system, and the stoichiometric ratio of the complex is given by the ratio of the excess solid acid present at the onset of precipitation to the caffeine consumed over the plateau length. The subsequent decrease in solubility is caused by the conversion of free acid in solution into a precipitated complex as further caffeine is added. Chemical analysis of precipitates separating in this plateau region indicated stoichiometric ratios of 2·6 : 1 and 2·9 : 1 (caffeine : acid), whilst the value calculated from the plateau of the phase diagram was 2·45 : 1. However, the linearity of the initial part of the diagram is positive evidence *against* the formation of a complex with more than one molecular proportion of caffeine, and

a constant K value (average 34·9) was only obtained on the assumption of a 1 : 1 reaction in solution. The stability constant K was also calculated from the slope of the rising portion in the following manner:

For the reaction: caffeine (C) + acid (A) $\rightleftharpoons$ complex (CA),

$$K = \frac{[CA]}{[C] \times [A]}$$

If A_o is the solubility of the acid in the solvent

A_t is the stoichiometric concentration of acid, i.e., [CA] + [A]

C_t is the stoichiometric concentration of caffeine, i.e., [CA] + [C]

$$\text{then } K = \frac{A_t - A_o}{(C_t - A_t + A_o)\,A_o} \qquad \ldots\ \ldots\ \ldots\ \ldots \qquad (1)$$

$$KA_o\,(C_t - A_t + A_o) = A_t - A_o$$

$$A_t + KA_oA_t = KA_oC_t + KA_o^2 + A_o$$

$$A_t(1 + KA_o) = C_t(KA_o) + A_o(1 + KA_o)$$

$$A_t = \frac{KA_o}{1 + KA_o}\,C_t + A_o$$

If A_t is plotted against C_t, the slope $S = KA_o/1 + KA_o$

$$\text{hence } K = S/(1 - S)A_o \qquad \ldots\ \ldots\ \ldots \qquad (2)$$

The value thus obtained 34·7 moles^{-1} litre is in good agreement with the mean calculated K.

The divergence between the 3 : 1 ratio of the solid and the 1 : 1 ratio from equilibrium calculations may be explained either by the formation of mixed crystals or by pseudo "salting-out" of the caffeine by the acid. It is interesting to note that salting-out effects have been observed by Chambon (1937) and Gusyakov (1959) when certain salts were added to caffeine. In the present case, the concentration of acid is probably too low for such an effect to operate, nor indeed would salting-out effects give definite stoichiometric ratios. In view of the relative constancy of these, the theory of mixed crystal formation is preferred.

p-*Methoxybenzoic acid.* The phase diagram shown in Fig. 1 differs from that of the *ortho* isomer in that the plateau is caused by saturation of the solution with respect to caffeine. From this point, the solid phase is a mixture of excess acid with increasing quantities of solid caffeine. The initial linear slope implies the formation of a soluble complex of caffeine with acid mononuclear with respect to the caffeine (Rossotti & Rossotti, 1961). From the phase diagram we may calculate the stoichiometric ratio as the ratio of the complexed acid in solution to the complexed caffeine in solution (i.e. the difference between the total caffeine in solution at the precipitation point and the solubility of caffeine at 15°). By this method the caffeine : acid ratio is 2·88. This ratio did not yield a constant K value, whereas on a 1 : 1 basis, good agreement was obtained between the average calculated value and the above slope value (41·3, 42·3 moles^{-1} litre respectively).

o- *and* p-*nitrobenzoic acids.* The phase diagrams for the interaction of these with caffeine are shown in Fig. 2. The increase in solubility of

these is linearly related to the amount of caffeine added. The break in the curve is due to the saturation of the solution with respect to caffeine and the acid concentration beyond this point is invariant.

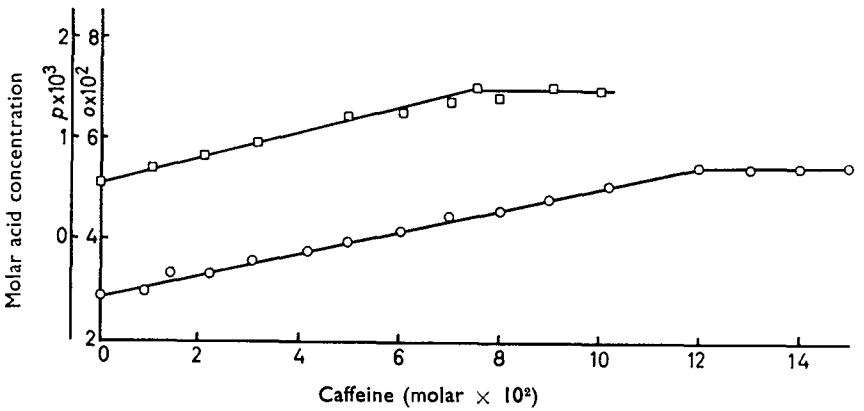

FIG. 2. Influence of caffeine on the solubilities of *ortho-* and *para-* nitrobenzoic acids. —○— = *ortho* in 0·0025 N HCl. —□— = *para* in 0·001 N HCl.

As in the previous case where the interaction product had a high solubility, the stoichiometric ratios could not be obtained from the phase diagrams, and the method used by Higuchi & Lack (1954) may yield misleading results in such systems. In the present study with *o-* and *p*-nitrobenzoic acids, the ratio (caffeine : acid) obtained corresponded to 1·9 and 6·02, respectively. The stability constant for the interaction of *o*-nitrobenzoic acid and caffeine calculated on a 2 : 1 (caffeine : acid) basis

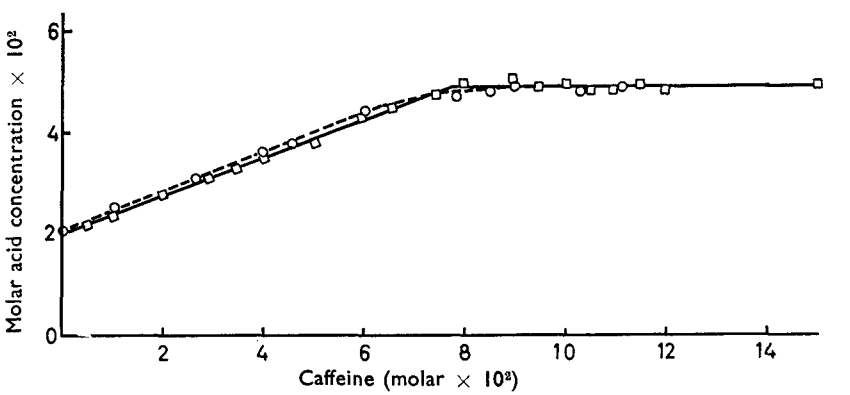

FIG. 3. Effect of caffeine on the solubility of benzoic acid at 15° C. - - -○- - - =solvent, 0·001 N HCl. —□— = solvent, water.

did not yield a constant value, but the assumption of a 1 : 1 reaction in both *ortho* and *para* acid-caffeine systems yielded K values which were in good agreement (*ortho*-acid, average K 9·92; slope method, 9·60; *para*-acid, average K 22·8; slope method 23·0, all values being in moles^{-1} litre).

A possible cause of the discrepancy may lie in the assumptions made, namely:

(i) The concentration of free acid is assumed to remain constant throughout and to be unaffected by the presence of the complexing agent.

(ii) The solubility of caffeine is assumed to remain unaltered in the presence of acid and complex.

Unfortunately a test of the accuracy of these assumptions is difficult to make although we have obtained some evidence that the solubility of caffeine is unaffected. Stoichiometric ratios have not been calculated for a number of soluble complexes by Higuchi & others, probably for the same reason.

Thermal analysis on *o*- and *p*-nitrobenzoic acid–caffeine systems has revealed no interaction (Sekiguchi, 1961).

Benzoic acid. The phase diagrams (Fig. 3) resemble those of the nitrobenzoic acids, the plateau being due to the precipitation of caffeine.

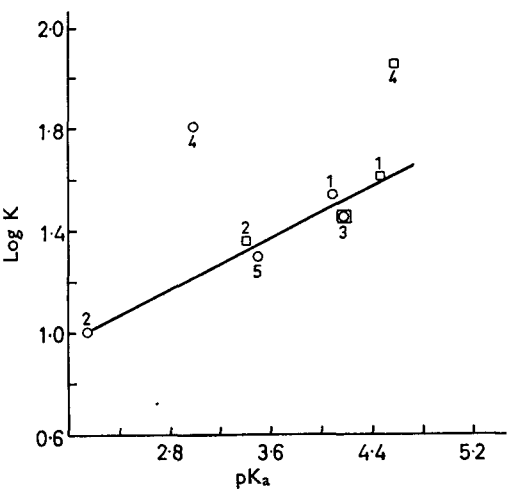

FIG. 4. Relation between log K and pK_a for substituted benzoic acids: 1. Methoxy. 2. Nitro. 3. Hydrogen. 4. Hydroxy. 5. Acetyloxy. ○ = *ortho* acids. □ = *para* acids.

The initial solubility of the organic acid in 0·001N hydrochloric is slightly higher than in water probably due to the greater polarity of the former solvent. With the addition of caffeine the solubility increase of benzoic acid in acid and in water is not identical, the slopes of the lines being 0·380 and 0·353, respectively. However, the maximum concentration attained in both the cases is the same.

The true stoichiometric ratio of the complex cannot be calculated from the phase diagram, but since the solubility increase is linear, a 1:1 caffeine:acid complex may be formed. K values in both solvents are reasonably constant on a 1:1 basis and the average values of 28·2 and 30·6 moles^{-1} litre in water and 0·001N hydrochloric acid respectively are

in good agreement with the values of K calculated from the slopes of the solubility phase diagrams (27·4 and 30·4 moles^{-1} litre, respectively).

This system has been studied by Higuchi & Zuck (1952, 1953) by the distribution method; two complexes 1:1 and 1:2 (caffeine:acid) were postulated. A 2:1 complex has also been detected in addition to 1:1 and 1:2 complexes by means of the interferometric technique (Donbrow & Jan, 1963). With solubility studies, a 1:2 complex is not easily detected if formed in small amount in solution in the presence of the 1:1 complex. It is interesting to note that the depression of ionisation results in non-linearity at high caffeine concentration when acid is used as a solvent; the solubility increase becomes less pronounced (Fig. 3).

Sekiguchi's (1961) studies of the melting point–composition diagram of the mixtures of benzoic acid and caffeine did not indicate any interaction.

STABILITY CONSTANTS AND STRUCTURE

Taken in conjunction with earlier data, the stability constants reported in this work enable a qualitative assessment to be made of the relative tendencies of substituted benzoic acids to form complexes with caffeine. Stability constants for 1:1 complexes are listed in Table 7. The values for the hydroxybenzoic acids were estimated from Higuchi's solubility data, and are approximate. It is apparent that the binding strength decreases in the order *p*-OH > *p*-OMe > H > *p*-NO_2, and *o*-OH > *o*-OMe > H > *o*-Ac > *o*-NO_2. The trend is in the order of increasing electronegativity of the substituent, and follows the order of the Hammett σ values of the *para* compounds (see Table 6; Jaffé, 1953).

TABLE 7. STABILITY CONSTANTS (K) FOR COMPLEXES OF CAFFEINE WITH SUBSTITUTED BENZOIC ACIDS R.C_6H_4·COOH (CALCULATED ON 1:1 BASIS AT 15°)

R	(moles^{-1} litre) K (mean) 1:1	log K	pK_a‡
o-OMe ..	34·9	1·54	4·09
p-OMe ..	41·3	1·62	4·47
o-NO_2 ..	9·9	1·00	2·17
p-NO_2 ..	22·8	1·36	3·44
H	28·2	1·45	4·20
o-OH ..	64*†	1·81	2·98
p-OH ..	115*†	2·06	4·58
o-OCOMe ..	19·8†	1·30	3·49

* Values estimated by present authors. † Higuchi & Zuck (1953). ‡ Braude & Nachod (1955)

The polar effects of substituents in benzoic acids is reflected in the pK_a values of the acids, and a plot of pK_a against log K indicates that there is an approximately linear relationship between these functions for all the acids with the exception of the hydroxybenzoic acids (Fig. 4). It is therefore probable that the same polar effects (i.e. a summation of inductive, resonance and steric effects) operate in complex formation as in ionisation, though without necessarily involving proton release. The linear trend implies a common mechanism throughout the series, and there are two main possibilities to be considered. One involves the carboxyl

group, which may interact with a particular part of the caffeine molecule either by means of electrostatic (dipole-dipole or dipole-induced dipole) forces or by a donor-acceptor mechanism. The second is concerned with the aromatic ring electrons, which may be involved in the formation of a donor-acceptor complex.

Direct involvement of the substituent group in the interaction is improbable however. If there were a direct substituent-caffeine link, one would expect to find a simple correlation between binding strength and a property of the substituent group such as dipole moment or polarisability; this is not the case. Indeed, the varying geometry and differing polar properties of the substituent groups would make a common specific mechanism involving these groups in the *ortho-* and *para-* positions highly improbable. The same argument militates against two-point attachment, which would not give a linear pK_a–logK plot. However, the deviation of the hydroxybenzoic acids is such that, for these acids, it is reasonable to postulate hydrogen-bonding as an additional binding mechanism.

Electron donor-acceptor mechanisms should cause changes in spectroscopic properties. Eckert (1962) has postulated such a mechanism, with the caffeine as acceptor, to account for new ultraviolet "charge-transfer" bands which he obtained in caffeine-procaine hydrochloride mixtures by differential methods. We have repeated his work and obtained similar bands using his and our own systems, but consider the bands to be spurious and experimental artifacts caused by excessive diminution of energy in the reference beam. From quantum mechanical calculations, Pullman (1958) considered that caffeine should be a good electron donor. The stability constants reported above would on the contrary support electron-acceptance by the caffeine and there is in fact an approximate correlation between the ionisation potentials of the monosubstituted benzenes (no data are available for the acids) and the log K values of the complexes. However, since the electron charge density in the carboxyl group may be similarly related to the ionisation potentials, the data do not distinguish between the mechanisms.

References

Braude, E. A. & Nachod, F. C. (1955). *Determination of Organic Structures by Physical Methods*, New York: Academic Press.
Chambon, M., Bouvier, J. & Duron, P. (1937). *J. Pharm. Chim.*, **26**, 216–231.
Donbrow, M. & Jan, Z. A. (1963). *J. chem. Soc.*, 3845–3850.
Eckert, T. (1962). *Arch. Pharm.*, **295**, 233–240.
Gusyakov, V. P. (1959). *Aptechno Delo*, **8**, No. 5, 30–33.
Higuchi, T. & Lack, J. L. (1954). *J. Amer. pharm. Ass., Sci. Ed.*, **43**, 349, 527.
Higuchi, T. & Zuck, D. A. (1952). *Ibid.*, **41**, 10–13.
Higuchi, T. & Zuck, D. A. (1953). *Ibid.*, **42**, 132–137.
Jaffé, H. H. (1953). *Chem. Rev.*, **53**, 191–261.
Labes, R. (1930). *Arch exp. Path. Pharmak.*, **158**, 42–52.
Pullman, A. & Pullman, B. (1958). *Proc. Nat. Acad. Sci., U.S.A.*, **44**, 1197–1202.
Rossotti, F. J. C. & Rossotti, H. (1961). *Stability Constants and other Equilibrium Constants in Solution.* London: McGraw Hill.
Sekiguchi, K. (1961). *J. pharm. Soc. Japan*, **80**, 669–674.