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Molecular interactions of caffeine with *o*-, *m*- and *p*-iodobenzoic acids and *o*-, *m*- and *p*-fluorobenzoic acids

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The solubilities of *o*-, *m*- and *p*-iodo- and fluorobenzoic acids in aqueous solutions of caffeine increase linearly with caffeine concentration due to complex-formation. Insoluble complexes are formed by the *o*- and *m*-acids and soluble ones by the *p*-acids. Approximate 1:1 stability constants have been evaluated from the phase diagrams and their values are considered in relation to possible mechanisms of complex-formation.

IN an initial examination on the nature of the complexation reaction between aromatic substances and caffeine, Donbrow & Jan (1965) outlined some of the problems of correlating binding strength and mechanism of complexation with structural features of the aromatic compound complexed. Apart from methodological restrictions, which originated from the absence of measurable changes in the physico-chemical properties of the molecules concerned, results have been difficult to interpret because of the variety of structures studied. Hence, earlier work (Labes, 1930; Higuchi & Zuck, 1952, 1953, 1954) has not led to a clear understanding of the phenomena involved. Donbrow & Jan stressed the need to examine closely-related series of complexants selected to limit the number of structural factors which could influence the binding. Using this approach, these authors showed that there was an approximately linear relationship between log K of the caffeine complex (where K = apparent 1:1 stability constant) and the pK_a value of the acid for six benzoic acids; the complexes of *o*- and *p*-hydroxybenzoic acids were exceptional, probably because hydrogen-bonding was additionally involved (see Higuchi & Zuck, 1953).

We here report the extension of the studies to another six non-hydrogen-bonding monosubstituted benzoic acids.

Experimental and results

SOLUBILITY

Excess of the organic acid was shaken at 15° with quantities of caffeine in aqueous acidic solution, an aliquot portion was filtered and the organic acid content determined titrimetrically or spectrophotometrically. In the spectrophotometric determination, the caffeine was extracted by shaking with chloroform, before the determination of the acid. The general technique was the same as described previously (Donbrow & Jan, 1965).

Quantities of acid used were: o-*Iodobenzoic acid* (125 mg) in 0·002N hydrochloric acid (50 ml). Titration indicator, cresol red. Results are in Table 1.

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TABLE 1. SOLUBILITY OF *o*-IODOBENZOIC ACID IN CAFFEINE SOLUTIONS CONTAINING 0·002N HYDROCHLORIC ACID AND *o*-FLUOROBENZOIC ACID IN CAFFEINE SOLUTIONS CONTAINING 0·025N HYDROCHLORIC ACID AT 15°

Caffeine conc. moles × 10^2	*o*-Iodobenzoic acid conc. moles × 10^3	K $moles^{-1}$ litre	Caffeine conc. moles × 10^2	*o*-Fluorobenzoic acid conc. moles × 10^3	K $moles^{-1}$ litre
0·00	1·86	—	0·00	2·99	—
1·00	2·29	24·2	0·00	3·05	—
1·10	2·34	24·5	1·04	3·45	25·4
2·00	2·79	26·2	1·52	3·84	41·2
2·60	3·04	25·6	2·33	4·29	41·3
3·00	3·32	27·5	3·27	4·73	37·1
3·62	3·28		3·38	4·85	40·3
4·00	3·26		3·51	4·74	32·8
5·00	3·29		3·97	5·07	36·4
5·28	2·89		4·35	5·10	31·1
6·00	2·49		4·73	5·25	32·2
7·00	1·92		5·43	5·60	30·6
7·92	1·42		6·57	6·10	30·6
9·30	1·25		7·45	6·80	34·7
10·00	1·22		7·79	6·65	
11·00	1·23		8·54	6·77	
12·00	1·22		9·90	6·95	
			10·30	6·80	
			10·65	6·00	
			11·75	5·15	
			12·30	4·80	
			13·30	4·70	
K av. = 25·6 K slope = 25·7			K av. = 34·5 K slope = 31·5		

m-*Iodobenzoic acid* (125 mg) in 0·002N hydrochloric acid (50 ml). Spectrophotometric determination λ_{max} 217 mμ; ϵ_{max} 11,150. Results are in Table 2.

p-*Iodobenzoic acid* (125 mg) in 0·002N hydrochloric acid (50 ml). Spectrophotometric determination λ_{max} 257 mμ; ϵ_{max} 14,725. Results are listed in Table 3.

TABLE 2. SOLUBILITY OF *m*-IODOBENZOIC ACID IN CAFFEINE SOLUTIONS CONTAINING 0·002N HYDROCHLORIC ACID AND OF *m*-FLUOROBENZOIC ACID IN CAFFEINE SOLUTIONS CONTAINING 0·025N HYDROCHLORIC ACID AT 15°

Caffeine conc. moles × 10^2	*m*-Iodobenzoic acid conc. moles × 10^4	K ($moles^{-1}$ litre)	Caffeine conc. moles × 10^2	*m*-Fluorobenzoic acid conc. moles × 10^2	K ($moles^{-1}$ litre)
0·00	5·38	—	0·00	1·09	—
0·48	5·90	20·3	0·95	1·28	22·9
1·08	7·00	28·3	1·05	1·34	28·7
1·84	8·00	26·9	1·60	1·48	29·6
2·31	9·40	32·9	1·75	1·54	31·8
3·14	10·81	32·7	2·20	1·50	21·0
3·93	11·60	29·9	2·70	1·70	26·8
4·65	13·30	31·8	3·20	1·76	24·3
5·00	13·40	30·3	3·50	1·82	24·2
5·40	13·50		3·57	1·83	
6·25	13·30		4·10	1·85	
7·12	13·80		4·60	1·84	
7·41	13·50		4·73	1·87	
7·80	13·40		4·75	1·70	
8·30	12·30		5·00	1·16	
8·70	11·40		5·27	0·98	
9·20	10·40		5·50	0·98	
10·00	9·00		5·70	0·98	
			6·00	0·96	
			6·95	0·87	
			8·69	0·82	
			9·05	0·87	
			11·90	0·87	
K av. = 29·1 K slope = 30·2			K av. = 26·1 K slope = 24·5		

TABLE 3. SOLUBILITY OF *p*-IODOBENZOIC ACID IN CAFFEINE SOLUTIONS CONTAINING 0·002N HYDROCHLORIC ACID AND OF *p*-FLUOROBENZOIC ACID IN CAFFEINE SOLUTIONS CONTAINING 0·025N HYDROCHLORIC ACID AT 15°

Caffeine conc. moles × 10^2	*p*-Iodobenzoic acid conc. moles × 10^4	K (moles^{-1} litre)	Caffeine conc. moles × 10^2	*p*-Fluorobenzoic acid conc. moles × 10^3	K (moles^{-1} litre)
0·00	1·12	—	0·00	4·00	—
0·80	1·80	83·3	0·83	4·50	16·0
1·49	2·40	77·4	1·48	5·00	18·1
1·76	2·65	78·3	2·46	5·50	16·2
2·80	3·35	76·4	3·19	6·00	16·7
3·68	4·10	72·9	4·40	6·60	15·1
4·30	4·60	75·8	6·07	7·10	17·6
4·60	5·00	76·0	7·00	8·00	17·2
5·80	5·80	72·6	8·04	8·50	14·8
6·30	6·30	74·0	8·60	9·00	
6·41	6·46	75·0	8·90	8·85	
6·80	6·80	75·2	10·00	8·90	
7·20	7·00	73·5	10·50	9·00	
8·00	6·90		11·00	9·00	
9·60	6·90		11·50	9·00	
10·80	6·80				
	K av. = 75·5 K slope = 74·3			K av. = 16·5 K slope = 15·6	

o-*Fluorobenzoic acid* (250 mg) in 0·025N hydrochloric acid (25 ml). Titration indicator, phenol red. Results are listed in Table 1.

m-*Fluorobenzoic acid* (100 mg) in 0·025N hydrochloric acid (25 ml). Determination as previous acid. Results are listed in Table 2.

p-*Fluorobenzoic acid* (100 mg) in 0·025N hydrochloric acid (25 ml). Determination as previous acid. Results are listed in Table 3.

Point by point stability constants were calculated as described by Higuchi & Zuck (1952, 1953) and the mean value compared with the value obtained from the slope of the plot where the rising portion was linear (Donbrow & Jan, 1965).

Discussion

o-*Iodobenzoic acid.* Fig. 1 (●) shows the solubility curve. This has 3 stages and is typical of a system in which soluble complex formation is followed by precipitation of an insoluble complex over the plateau region, and then, when the excess solid acid is exhausted, there is a decrease in solubility due to the precipitation of complex at the expense of free acid in solution. Analysis of the solids separating in the region defined by the descending curve of Fig. 1 gave a molecular ratio of caffeine:acid of 3·4:1, whereas the calculated value from the parameters of the phase diagram was 2·5:1. However, the linearity of the initial stage indicates the formation of a mononuclear complex of caffeine (Rossotti & Rossotti, 1961); K values calculated from this stage on a 1:1 basis were: mean value 25·6, slope 25·6. The m.p. of the solid was 192–194°.

m-*Iodobenzoic acid.* The solubility curve is shown in Fig. 2 (●). It is similar to that of the *o*-iodobenzoic acid and can be interpreted in the same way. Chemical analysis of the solids isolated over the third stage gave a ratio of caffeine:acid of 3·5:1. The molecular ratio, calculated

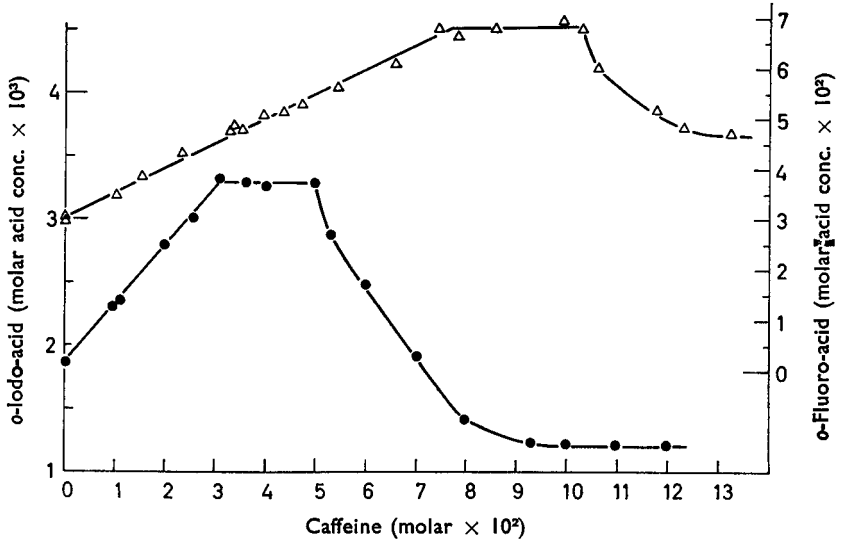

FIG. 1. Effect of caffeine on the solubility of *o*-iodo- and *o*-fluorobenzoic acid at 15° C. ●—●, Iodo-acid (solvent 0·002N HCl); △—△, fluoro-acid (solvent 0·025N HCl).

from the plateau is 2·8 : 1 and the 1 : 1 K values were 29·1 (mean) and 30·2 (slope). The m.p. of the solid was 142–144°.

p-*Iodobenzoic acid.* The solubility curve is shown in Fig. 3 (●). This acid differs from the other iodobenzoic acids in forming a complex that remains in solution. The plateau corresponds to saturation of the system with caffeine. From this point the solid phase is a mixture of excess acid with increasing amounts of solid caffeine. The initial linear

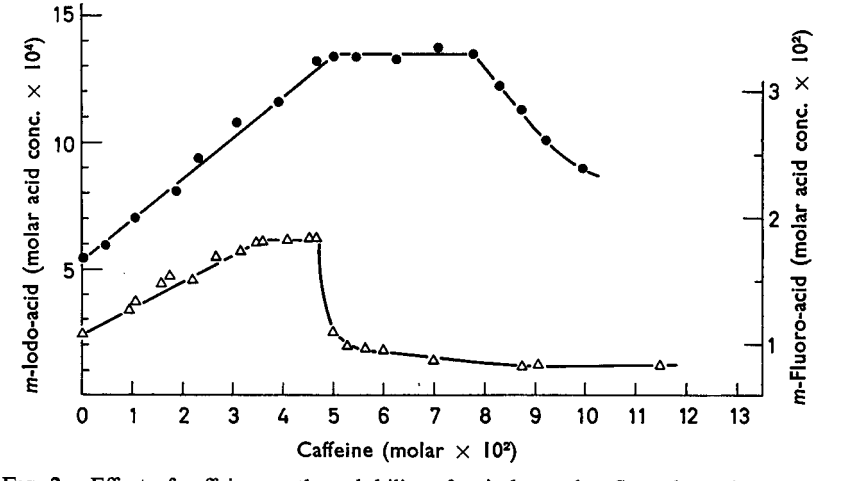

FIG. 2. Effect of caffeine on the solubility of *m*-iodo- and *m*-fluorobenzoic acids at 15° C. ●—●, Iodo-acid (solvent 0·002N HCl); △—△, fluoro-acid (solvent 0·025N HCl).

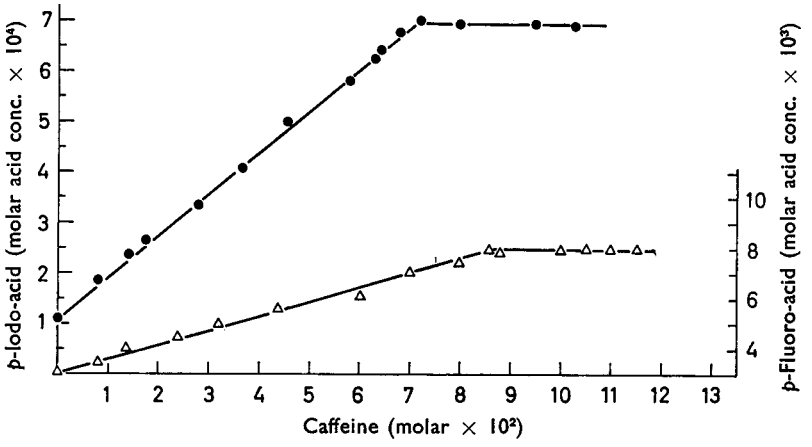

FIG. 3. Effect of caffeine on the solubility of *p*-iodo- and *p*-fluorobenzoic acids at 15° C. ●—●, Iodo-acid (solvent 0·002N HCl); △—△, fluoro-acid (solvent 0·025N HCl).

slope implies formation of a soluble complex mononuclear with respect to the caffeine. From the solubility increase of the caffeine and the acid, it was found that 6·2 moles of caffeine were solubilized for one mole of acid. Calculation of the K values on this basis did not give constant values whereas on a 1:1 basis values were virtually constant at 75·5 (mean) and 74·3 (slope).

o-*Fluorobenzoic acid.* The shape of the solubility curve, which is shown in Fig. 1 (△), follows the general pattern for insoluble complexes. Analysis of the solid compounds isolated in the region defined by the descending curve gave a molecular ratio of caffeine : acid of 1·2 : 1,whereas the ratio calculated from the plateau region was caffeine : acid 1·56 : 1. The m.p.'s of the solids were constant (105–108°). K values, calculated on a 1 : 1 basis, tended to fall with increase in the caffeine concentration, possibly because of the formation of higher complexes of caffeine. The mean 1 : 1 K value was 34·5 compared with 31·5 by the method of least squares.

m-*Fluorobenzoic acid.* The solubility curve is shown in Fig. 2 (△). This acid differs from the other halogen-substituted benzoic acids in showing a sharp decrease in the solubility of the acid after the plateau region. Chemical analysis of the solids precipitate gave a ratio for caffeine : acid of 1 : 1 compared with the plateau value of 1·2 : 1. Calculated on a 1 : 1 basis the K value from the slope was 24·4 and the mean value was 26·6. The m.p. of the solid was 119–121°.

p-*Fluorobenzoic acid.* The solubility surve, shown in Fig. 3 (△) is like that for the *p*-iodo acid and indicates the formation of a soluble complex. Although 2·3 moles of caffeine were solubilized per mole of acid, relatively constant K values were obtained on a 1 : 1 basis (mean 16·5, slope 15·6).

The high stoichiometric ratios observed with certain of these acids resemble those obtained in the earlier work. Were multinuclear caffeine complexes formed to any appreciable extent in solution, the solubility increase would show as a pronounced upward curvature with increasing caffeine concentration instead of the linearity actually found. Furthermore, the K values calculated on a 1 : 1 basis should be dependent on a power of the caffeine concentration because of reactions such as:

$$CA + C \leftrightharpoons C_2A$$
$$C_2A + C \rightleftharpoons C_3A, \text{ etc.}$$

(where C = caffeine; A = acid)

Since the curves and K values show no evidence of this, it must be concluded that the higher complexes make little contribution to the solubility increases.

The separation of insoluble higher complexes could be due to the formation of mixed crystals or inclusion compounds, or the very low solubility of the higher complexes, which would then precipitate in the presence of much higher concentrations of the lower complexes.* However, because of the instability of these higher complexes, their solubilities in water could not be measured.

The linearity of the curves does not preclude formation of a 1 : 2 complex, but in the iodo-acids, the low solubility, and hence low free acid concentration, is unfavourable to this. With the more soluble fluoro-acids, the validity of the 1 : 1 values is questionable but the values give a qualitative indication of the overall complexation which occurs.

For the iodo-acids, the binding strength decreases in the order *para* > *meta* > *ortho*, as in most of the benzoic acids studied hitherto, whereas the order is reversed in the fluoro-acids. Together with the previous data obtained at 15° (Donbrow & Jan, 1965), the apparent 1 : 1 constants decrease in the order: *para*, OH > I > OMe > H > NO_2 > F; *ortho*, OH > OMe ≈ F > H > I > OCOMe > NO_2. The values for the *m*-iodo- and *m*-fluoro-acids lie close to the line relating log K to pK_a of the acid (Fig. 4, Donbrow & Jan, 1965); this is not so for the other four acids. The *p*-fluoro-acid has an unexpectedly low constant and the *o*-fluoro- and *p*- and *o*-iodo-acids have unexpectedly high constants, the last being particularly surprising, since steric hindrance to the formation of a sandwich-type complex might have been anticipated; the projection of the iodine atom (van der Waals' radius 2·15 Å) outside the width of the aromatic ring (van der Waals' half-thickness 1·85 Å, see Pauling, 1960) not only fails to inhibit binding, but may possibly be associated with enhancement, which could be explained, in the three acids, by postulating either a different mechanism altogether, or a two-point attachment in which the "normal" interaction forces are supplemented by dipolar, induced dipolar or some other interaction connected with the halogen substituent. The same effect of enhancement of stability constant by *p*-iodo and weakening by *p*-fluoro has been observed by Andrews &

* "Salting-out" effects are unlikely to occur at such low concentrations of non-ionized substances.

Keefer (1949, 1950) in the complexation of substituted benzenes with aqueous silver ion, postulated as occurring by π-electron donation. The halogens do not behave regularly in a number of respects, and a plot of the ionization potentials of substituted benzenes against Taft's (1957) σ_+ constant for the *para*-substituent also shows the same kind of deviation, the iodobenzene having a much lower ionization potential and the fluorobenzene a higher one than expected (Streitweiser, 1963). This would accord with one of the theories put forward previously (Donbrow & Jan, 1965), that a donor-acceptor mechanism involving the aromatic ring electrons might be involved; the present evidence is in favour of the aromatic system as the donor. However, though there are still insufficient data to confirm the mechanism of complexation, it is apparent that complexation with caffeine is much enhanced in a number of benzoic acids substituted with mesomeric electron-donating groups, and weakened by electron attracting groups.

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