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Spectroscopic Studies on Molecular Interactions. I. Complexations between Caffeine and Benzoic Acids

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Interactions between caffeine and 21 un– and mono–substituted benzoic acids were investigated at pH 7.0 and 30° by means of an indirect method utilizing the spectral change due to the competition between congo red and benzoates in complexation with caffeine. The main molar composition of the complexes appeared to be 1:1, and apparent equilibrium constant and free energy change for the complex formation were evaluated. The relation between the free energy change and pK_a for benzoates suggested that direct electrostatic forces between the carboxyl group in the benzoate molecules and the nitrogen of the 7-position in the caffeine molecule played a dominant role in the complexations.

Caffeine complexes have long been a subject of pharmaceutical interest since the origination of caffeine sodium benzoate in the last centrury. But weak complexation in aqueous solution such as caffeine–benzoic acid system usually does not appear to be accompanied by changes in pH or spectra sufficient for the use of the usual direct techniques,[2] and caffeine complexes have been studied with a few kinds of acids mainly by solubility method or distribution method.[3] And the conclusions have not always been of one accord with regard to the mechanism of the complex formation, or even about occurring of the complexations.[4]

In the present work, equilibria between caffeine and 21 un– and mono–substituted benzoic acids were investigated at pH 7.0 by means of an indirect method utilizing the spectral change due to the competition between congo red and benzoates in complexation with caffeine to elucidate the mechanism of the interacions.

Experimental

Materials——Caffeine (J.P., Sanko Seiyaku Kogyo Co.) after recrystallization from water had mp 238°. All benzoic acids and congo red were of Guaranteed Reagent Grade, Tokyo Kasei Kogyo Co., and checked their melting points and spectra. All the test solutions were prepared with 0.1 M phosphate buffer solution of pH 7.0 and adjusted their pH with NaOH or HCl, if necessary. For the benzoic acids, an equimolar amount of NaOH was added in the preparation of the solutions of their Na salts. The initial concentrations of congo red and caffeine in the test solutions were 1×10^{-4} M and 1×10^{-2} M, respectively, if not especially described.

Measurements of Optical Absorption——Optical absorbance and spectra were measured in 10 mm cells at 20°, 30°, or 40° with a Hitachi-Perkin Elmer model 139 spectrophotometer, or at room temperature (24—27°) with a Hitachi model EPS-3 recording spectrophotometer about 24 hours after preparing the test solutions in duplicate.

1) Location: *1–5–8, Hatanodai, Shinagawa–ku, Tokyo.*
2) M. Donbrow and Z.A. Jan, *J. Chem. Soc.*, **1963**, 3845.
3) A brief review is appeared in A.N. Martin, "Physical Pharmacy," Lea & Febiger, Philadelphia, 1960, p. 405.
4) K. Sekiguchi, *Yakugaku Zasshi*, **81**, 664 (1961).

Results and Discussion

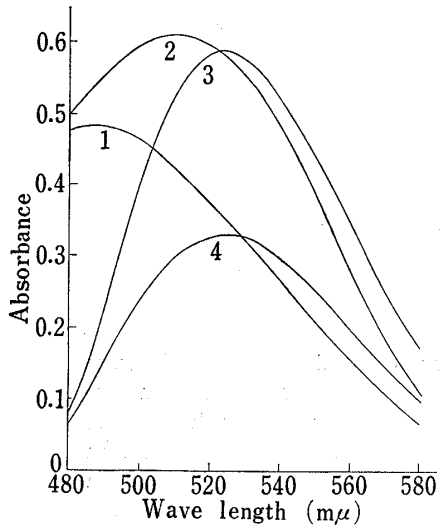

Fig. 1. Spectra of Mixed Solutions of Congo Red with Caffeine and Sodium Benzoate

1: congo red (2.5×10^{-5}M)
2: congo red (2.5×10^{-5}M) and caffeine (4.5×10^{-2}M)
3: congo red (9×10^{-5}M) and caffeine (1×10^{-2}M) *vs.* congo red
4: congo red (9×10^{-5}M), caffeine (1×10^{-2}M) and Na benzoate (9×10^{-2}M) *vs.* congo red

Equilibrium between Caffeine and Congo Red

Spectra of mixed solutions of congo red with caffeine and sodium benzoate were recorded in Fig. 1. It can be seen that the absorption of congo red is developed at about 525 mμ in the presence of caffeine, and that the spectral change is partially recovered by the addition of sodium benzoate. The caffeine–induced spectral change may be due to the complexation of congo red with caffeine, and the spectral recovery is supposed to be due to the competition of congo red with benzoate in complexation with caffeine.[5] Accordingly it may be expected that the interactions between caffeine and benzoates can be investigated by utilizing the competition. At the first step in the investigation, equilibrium between caffeine and congo red was studied.

The equilibrium in which a single molecule of congo red, A, may combine with n molecules of caffeine, B, is expressed as the following form

$$A+nB \rightleftarrows AB_n \qquad (1)$$

The equilibrium constant, K_A, is shown as

$$K_A=x/(a-x)(b-nx)^n \qquad (2)$$

where a and b are the initial concentration of A and B, respectively, and x is the concentration of A combined with B. The development of the optical absorption induced by caffeine may be expressed by

$$\Delta E=\Delta\varepsilon dx \qquad (3)$$

where ΔE is the difference between the absorbances at 525 mμ of congo red solutions in the presence and in the absence of caffeine, $\Delta\varepsilon$ the difference between the molar extinction coefficients at 525 mμ of combined and free congo red, and d the depth of the optical path. When $a\ll b$, Eq. (2) and (3) lead the following equation.

$$a/\Delta_E=1/\Delta\varepsilon b^n dK_A+1/\Delta\varepsilon d \qquad (4)$$

If $n=1$, Eq. (4) is identical with the Benesi–Hildebrand equation.[6]

Fig. 2 shows the plots of $a/\Delta E$ *versus* $1/b^n$ with $n=1/2$, 1, and 2 from the data of the equilibrium at 20°, 30°, and 40°. From Fig. 2, it is seen that a straight–line relationship is approximately satisfied between $a/\Delta E$ and $1/b^n$ only in the case of $n=1$ and at 30°, and it is considered that the main molar compositions of congo red–caffeine complex may be 2:1 and 1:1 at 20°, 1:1 at 30°, and 1:1 and 1:2 at 40°. The apparent values of $\Delta\varepsilon$ and K_A at 30° were estimated to be 1.45×10^4 and 84.6 liter/mole, respectively, from the graph.

5) It was observed that direct effect of benzoates on the spectrum of congo red was almost negligible.
6) H.A. Benesi and J.H. Hildebrand, *J. Am. Chem. Soc.*, **71**, 2703 (1949).

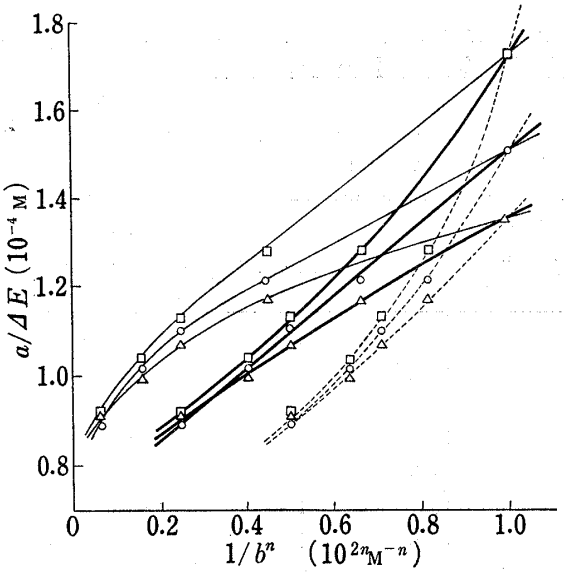

Fig. 2. Relation of $a/\Delta E$ to $1/b^n$ for Interaction between Caffeine and Congo Red

1×10^{-2}—4×10^{-2}M caffeine and 1×10^{-4}M congo red at 20° (△), 30° (○), and 40° (□)
——: $n=2$ ——: $n=1$ ------: $n=1/2$

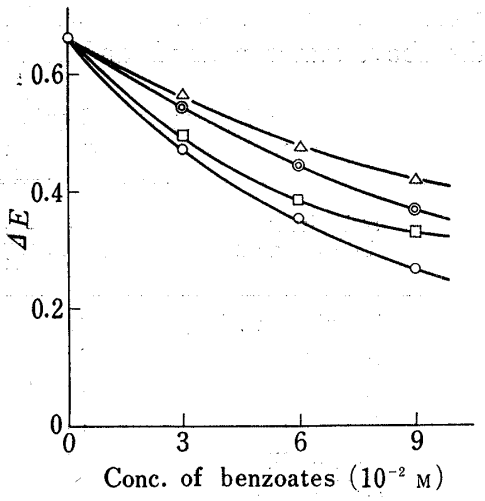

Fig. 3. ΔE of Congo Red–Caffeine Solutions with Varying Concentrations of Na Benzoate and Na Chlorobenzoates at 30°

1×10^{-4}M congo red and 1×10^{-2}M caffeine with 0—9×10^{-2}M benzoate (◎) and *ortho-* (△), *meta-* (○), and *para-* (□) chlorobenzoates

Interactions between Caffeine and Benzoates

Assuming that the main molar composition of caffeine–benzoate complexes is 1:1[7] at 30° as well as that of congo red–caffeine complex and that congo red and benzoates compete in the combining with caffeine in the mixed solutions, the equilibrium constants for complex formation of caffeine with congo red, K_A, and with benzoates, K_C, are shown in the competitive complexation as

$$K_A=x/(a-x)(b-x-y) \tag{5}$$

$$K_C=y/(c-y)(b-x-y) \tag{6}$$

where c is the initial concentration of benzoates, and y the concentration of caffeine–benzoate complexes. From Eq. (5) and (6), K_C is given by

$$K_C=K_A(a-x)y/x(c-y) \tag{7}$$

The value of x can be calculated by Eq. (3) if ΔE is known, and from Eq. (5) y may be given by

$$y=(b-x)-x/(a-x)K_A \tag{8}$$

Consequently, since the values of a, b, and c are given and the values of K_A and $\Delta\varepsilon$ are known, K_C can be evaluated by measurement of ΔE with the mixed solutions of congo red with caffeine and benzoates.

Fig. 3 shows some examples of ΔE values *versus* the concentration of benzoates added to a solution containing congo red and caffeine at 30°. The extent of the reversion of the caffeine–induced spectral change of congo red increases with the increasing concentration of benzoates added, and this may suggest the occurrence of the competition between congo red and benzoates in complexation with caffeine.

7) This assumption may be reasonable because the main molar composition of caffeine–acid complexes has been reported to be usually 1:1 (T. Higuchi and D.A. Zuck, *J. Am. Pharm. Assoc.*, **42**, 138 (1953)).

The values of log K_c and apparent free energy change, ΔG, for the complex formation between caffeine and benzoates were calculated from the observed values of ΔE by means of the above-stated method, and listed in Table I. Table I indicates that the values of log

Table I. Apparent Equilibrium Constant and Free Energy Change for Complexations between Caffeine and Benzoic Acids at pH 7.0 and 30°

Substituent of benzoic acids	log K_C Initial conc. of acids 3×10^{-2}M	6×10^{-2}M	9×10^{-2}M	Mean	ΔG (kcal/mole)
Unsubstituted	1.14	1.22	1.26	1.21	−1.68
o-Amino	0.92	1.27	1.26	1.15	−1.60
m-Amino	1.26	1.07	1.22	1.18	−1.64
p-Amino	1.04	1.10	1.22	1.12	−1.55
o-Hydroxy	1.67	1.67	1.50	1.61	−2.23
m-Hydroxy	1.38	1.43	1.44	1.42	−1.97
p-Hydroxy	1.25	1.36	1.46	1.36	−1.89
o-Methoxy	0.78	0.90	0.90	0.86	−1.19
p-Methoxy	1.38	1.40	1.35	1.38	−1.91
o-Methyl	0.90	0.91	0.94	0.92	−1.28
m-Methyl	1.29	1.34	1.36	1.33	−1.85
p-Methyl	1.25	1.28	1.41	1.31	−1.82
o-Acetamino	1.59	1.56	1.43	1.53	−2.12
m-Acetamino	1.33	1.26	1.25	1.28	−1.78
p-Acetamino	1.22	1.32	1.23	1.26	−1.75
o-Chloro	0.84	0.94	0.95	0.91	−1.26
m-Chloro	1.44	1.47	1.53	1.48	−2.05
p-Chloro	1.41	1.40	1.32	1.38	−1.91
o-Bromo	0.92	1.05	1.05	1.01	−1.40
m-Bromo	1.49	1.54	1.52	1.52	−2.11
p-Bromo	1.54	1.48	1.43	1.48	−2.05

K_c are nearly irrespective of the initial concentrations, and this may justify the procedure and the assumptions involved in the above-mentioned method. And the justification may be also supported by the facts that correlation between the obtained values of ΔG and solubility of caffeine in the presence of benzoates[8] is highly significant with the coefficient of −0.95 (12 samples) as shown in Fig. 4, and that ΔG in kcal/mole has been estimated to be −1.80 for caffeine and benzoic acid,[7] −1.54 for caffeine and benzoate ion,[9] and −2.27 for caffeine and salicylic acid[7] in distilled water at 30° by solubility method.

A good linear-relationship between ΔG and pK_a[10] for the benzoates is observed in Fig. 5 except for a number of benzoates which have hydrophobic substituent in the *ortho*-position. This may suggest that direct electrostatic forces[11] play a dominant role in the 1:1 interactions, and that the carboxyl group in the benzoate molecules takes an important part in the complex formation. And this may support Chertkoff's suggestion[12] that in the caffeine molecule a relatively positive center, presumably the nitrogen of the 1-position, exists which serves as a likely site of the complexation. But the positive site is considered to be the nitrogen of the

8) The solubility was estimated from the graphs in ref. 4).
9) T. Higuchi and D.A. Zuck, *J. Am. Pharm. Assoc.*, **42**, 132 (1953).
10) A. Albert and E.P. Serjeant, "Ionization Constants of Acids and Bases," Methuen & Co., London, 1962.
11) J.O. Hirshfelder, "Molecular Biophysics," ed. by B. Pullman and M. Weissbluth, Academic Press, New York, 1965, p. 325.
12) M. Chertkoff, personal communication, through ref. 3), p. 406.

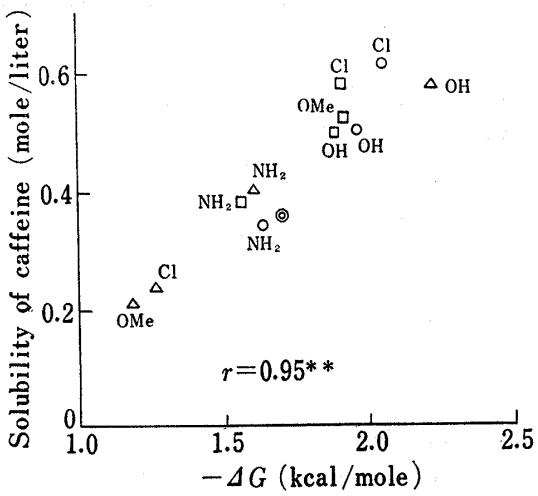

Fig. 4. Correlation between $-\Delta G$ for the Complexation and Solubility of Caffeine in the Presence of Benzoates (Na Salts, 0.4 M) in Distilled Water at 25°

◎: benzoate △: *ortho*-substituted benzoates
○: *meta*-substituted benzoates
□: *para*-substituted benzoates
** highly significant at the 1% level

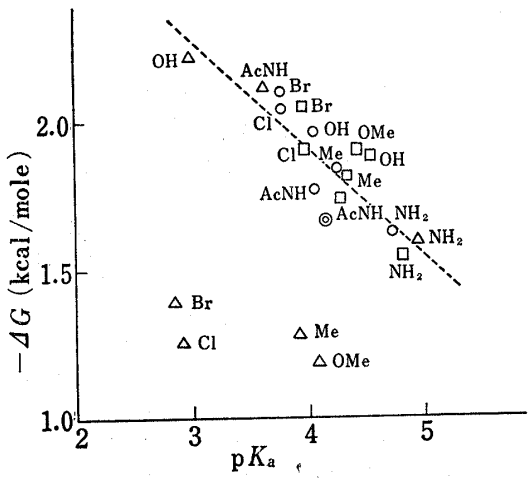

Fig. 5. Relation between $-\Delta G$ for the Complexation and pK_a of Benzoates

◎: benzoate △: *ortho*-substituted benzoates
○: *meta*-substituted benzoates
□: *para*-substituted benzoates

7-position, and not of the 1-position, according to calculated π–electron distributions of caffeine molecule[13] and of xanthine molecule.[14]

Besides the direct electrostatic forces several minor forces such as stacking, hydrogen bonding, and charge–transfer forces may probably contribute to the complex formation, but these are not cleared from the results of this work.

13) R.S. Schnaare and A.N. Martin, *J. Pharm. Sci.*, **54**, 1707 (1965); S. Hata, K. Mizuno, and S. Tomioka, *Chem. Pharm. Bull.* (Tokyo), **16**, 1 (1968).
14) B. Pullman and A. Pullman, "Quantum Biochemistry," Interscience Publishers, New York, 1963, p. 843.