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Adsorption of Benzoic Acid Derivatives by Carbon Black from Aqueous Solution and Related Phenomena[1]

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The adsorbability of 22 *ortho*-, *meta*- and *para*-mono- or-disubstituted benzoic acid derivatives by carbon black from aqueous solution (pH 7.4) was correlated with that on graphite from solution (pH 7.0), but not with pK_a, partition coefficient and plain size of molecule.

The adsorbability of *o*-nitro, -methyl, -chloro and -bromobenzoic acids was less than that of the respective *metha*- and *para*-substituted compounds. The thermodynamic functions for *ortho*-substituted benzoic acids also were different from those for *meta*- and *para*-substituted ones, except for amino- and hydroxybenzoic acids.

Plotting the solubility of caffein in the solutions containing benzoic acid derivatives and the free energy change of complex formation with caffein, respectively, against the adsorbability of the acid derivatives on carbon black, there were found correlations in both cases without excepting for *ortho*-substituted ones. A linear relationship was observed between the free energy change of binding to serum albumin and the adsorbability on carbon black of benzoic acid derivatives without excepting for *ortho*-substituted ones. These two results suggested that both the complex formation with caffein and the protein binding of benzoic acid derivatives take place on the basis of the electrostatic and the hydrophobic interactions.

In this series of works,[3–6] it has been elucidated that the adsorption of organic medicinals by carbon black proceeds on the basis of the hydrophobic interaction and thus the adsorbability on carbon black corresponds to the hydrophobicity of the respective medicinals. Then, various discussions have been made as to the correlation between the adsorbability and the biopharmaceutical data reported.

Regarding benzoic acid derivatives, there have been reported a variety of pharmaceutically interesting data, *e.g.*, pK_a,[7] partition coefficient,[8] complex formation with caffein,[9,10] and binding to bovine serum albumin.[11] Moriguchi, *et al.* investigated the adsorption of 14 *para*- and *meta*-monosubstituted benzoic acid derivatives on graphite from solution (pH 6.0, 7.0 and 8.0).[12]

In the present study, the adsorption of 22 *ortho*-, *meta*- and *para*-mono or disubstituted benzoic acid derivatives by carbon black from solution (pH 7.4) was investigated in detail

1) This paper forms Part XVIII of "Physico-chemical Approach to Biopharmaceutical Phenomena." Preceding paper, Part XVII: T. Oguma, T. Nagai, and H. Nogami, *Chem. Pharm. Bull.* (Tokyo), **19**, 124 (1971).
2) Location: *Hongo, Bunkyo-ku, Tokyo.*
3) H. Nogami, T. Nagai, and H. Uchida, *Chem. Pharm. Bull.* (Tokyo), **17**, 176 (1969).
4) H. Nogami, T. Nagai, and S. Wada, *Chem. Pharm. Bull.* (Tokyo), **18**, 348 (1970).
5) H. Nogami, T. Nagai, and N. Nambu, *Chem. Pharm. Bull.* (Tokyo), **18**, 1643 (1970).
6) N. Nambu, T. Nagai, and H. Nogami, *Chem. Pharm. Bull.* (Tokyo), in press.
7) A. Albert and E.P. Serjeant, "Ionization Constants of Acids and Bases," Methuen *w* Co., Ltd., London, 1962.
8) C. Hansh and T. Fujita, *J. Am. Chem. Soc.*, **86**, 1616 (1964).
9) K. Sekiguchi, *Yakugaku Zasshi*, **81**, 664 (1961).
10) I. Moriguchi, M. Nayuki, and N. Kaneniwa, *Chem. Pharm. Bull.* (Tokyo), **17**, 1939 (1969).
11) I. Moriguchi, *Chem. Pharm. Bull.* (Tokyo), **16**, 597 (1968).
12) I. Moriguchi, S. Fushimi, and N. Kaneniwa, *Chem. Pharm. Bull.* (Tokyo), **18**, 449 (1970).

and discussions were made in comparison with the existing data mentioned above. Especially, *ortho*-substituted derivatives were included here, which had not been included in the report of the adsorption of graphite by Moriguchi, *et al.*[12]

Experimental

Materials——Carbon black marketed as "Seisei Shirasagi" by Takeda Chemical Ind. was used after the same treatment as described in a previous paper.[13] The rest of the materials were of the purest reagent grade. *p*-Hydroxybenzoic acid was used after heating at 100° for 24 hr.[14]

Procedure for Determination of the Adsorbed Amount by Batch Method——Fifty mg of carbon black was added in 10 ml of the respective compounds in 1/15M phosphate buffer solution (pH 7.4) at 17° or 37°, and then the procedure was carried out in the same way as described in the previous paper.[13]

Quantitative Determination of Benzoic Acid Derivatives——After diluting the sample with the same buffer solution as used for the adsorption experiment mentioned above, the concentration of the compound was determined according to ultraviolet (UV) absorption method using a Hitachi 124 spectrophotometer: benzoic acid at 224 mμ; *o*-nitrobenzoic acid, 268; *m*-nitrobenzoic acid, 266; *p*-nitrobenzoic acid, 272; *o*-aminobenzoic acid, 307; *m*-aminobenzoic acid, 300; *p*-aminobenzoic acid, 266; *o*-methoxybenzoic acid, 279; *p*-methoxybenzoic acid, 247; *o*-methylbenzoic acid, 228; *m*-methylbenzoic acid, 228; *p*-methylbenzoic acid, 234; *o*-chlorobenzoic acid, 230; *m*-chlorobenzoic acid, 224; *p*-chlorobenzoic acid, 234; *o*-bromobenzoic acid, 220; *m*-bromobenzoic acid, 220; *p*-bromobenzoic acid, 239; *o*-hydroxybenzoic acid, 295; *m*-hydroxybenzoic acid, 287; *p*-hydroxybenzoic acid, 245; 2,4-dichlorobenzoic acid, 227.

Result and Discussion

The absorption isotherms obtained for all the samples of benzoic acid derivatives were well described with Langmuir equation (1), for example, as shown in Fig. 1.

$$1/M = 1/a + 1/abC \qquad (1)$$

where M is the amount adsorbed at the concentration C in solution at equilibrium, a the amount adsorbed when the entire surface is covered by a monolayer, and b the equilibrium constant of adsorption process.

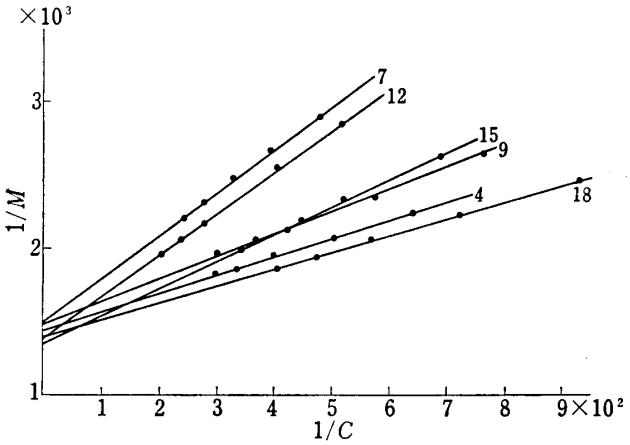

Fig. 1. Examples of Langmuir Plots of Adsorption of Benzoic Acid Derivatives by Carbon Black at 37° represented by the Same No. as in Table I

C: equilibrium concentration (M)
M: adsorbed amount (mole/g)

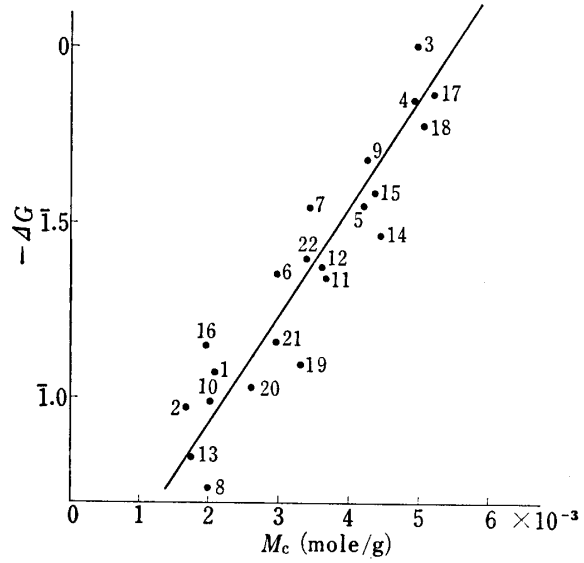

Fig. 2. Plot of the Free Energy Change of Adsorption, ΔG, against the Adsorbed Amount at Equilibrium Concentration 2×10^{-3}M, M_c, of Benzoic Acid Derivatives by Carbon Black at 37° represented by the Same No. as in Table I

13) H. Nogami, T. Nagai, E. Fukuoka, and H. Uchida, *Chem. Pharm. Bull.* (Tokyo), **16**, 2248 (1968).
14) H. Nogami, T. Nagai, and T. Yotsuyanagi, *Chem. Pharm. Bull.* (Tokyo), **17**, 499 (1969).

Moriguchi, *et al.*[12] expressed the adsorbability by log *ab* which corresponds to the logarithm of the first equilibrium constant in the multiple equilibria proposed by Klotz, *et al.*[15] In the present study, plotting ΔG ($=-RT\ln ab$) against the adsorbed amount at an identical equilibrium concentration, M_c, a linear relationship was given as shown in Fig. 2, where the equilibrium concentration was 2×10^{-3}M. M_c generally is more acculately obtainable than log *ab*, being suitable to comparing the adsorbability among benzoic acid derivatives. Therefore, M_c will be applied to the following discussions.

Relationship between the Adsorptions of Benzoic Acid Derivatives by Carbon Black and by Graphite[12]

The adsorbed amount M_c of *meta*- and *para*-mono substituted benzoic acids by carbon black was correlated with the data of log *ab* in the case of graphite at pH 7.0 reported by Moriguchi, *et al.*,[12] as shown in Fig. 3. *ortho*-Benzoic acid derivatives are supposed to give the same result, though there was no datum in the case of graphite.

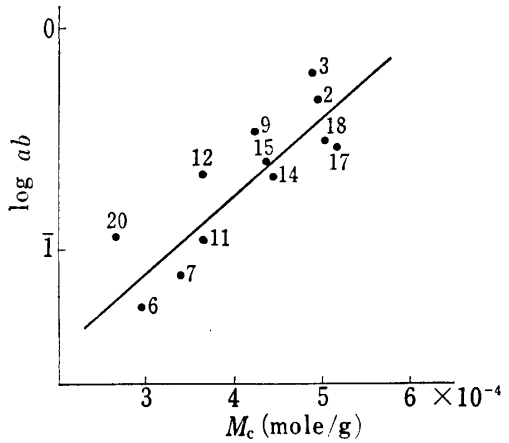

Fig. 3. Plot of log *ab* by Graphite at 5° Reported by Moriguchi, *et al.*[12] against the Adsorbed Amount at Equilibrium Concentration 2×10^{-3}M, M_c by Carbon Black at 37° of Benzoic Acid Derivatives Represented by the Same No. as in Table I

Since it is known that the surface of graphite is more homogeneous and more hydrophobic than that of carbon black, the correlation shown in Fig. 3 demonstrated that the adsorption of benzoic acid derivatives proceeds on the basis of hydrophobic interaction between the hydrophobic moiety of the adsorbate molecule and the carbon black surface. In other words, the carbon black is made a model of the hydrophobic surface, as was discussed in previous papers.[1,13,16]

Difference in Adsorbed Amount M_c with Regard to the Substituted Position of Benzoic Acid Derivatives

In the adsorption of phenols by carbon black from aqueous solution,[17] there was found no difference in adsorbed amount among *ortho*-, *meta*- and *para*-substituted compounds in undissociated state. However, in the present case, the adsorbed amounts of *o*-nitro, -methyl, -chloro, and -bromobenzoic acids were less than those of the respective *meta*- and *para*-substituted compounds, as shown in Table I.

The thermodynamic functions for *ortho*-substituted benzoic acids also were different from those for *meta*- and *para*-substituted ones, except for amino- and hydroxybenzoic acids.

The benzoic acid derivatives were almost in dissociated state in the present experimental conditions, and the *ortho*-substituent, even if hydrophobic itself, seemed to take no part in the adsorption, but to give effect on COO^- to increase its affinity to water or to decrease the affinity of the molecule to carbon dlack surface.

Fukui, *et al.* found a distinct correlation between the frontier electron distribution at the *ortho* position and the plant growth activity of benzoic acid derivatives, describing that the chemical reaction of these compounds at the *ortho* position with a nucleophilic group of plant

15) I.M. Klotz, F.M. Walker, and R.B. Pivan, *J. Am. Chem. Soc.*, **68**, 1486 (1946); I.M. Klotz, "The Protens," Vol. I, Academic Press, New York, 1953, p. 727.
16) H. Nogami, T. Nagai, and S. Wada, *Chem. Pharm. Bull.* (Tokyo), **18**, 342 (1970).
17) H. Umeyama, T. Nagai and H. Nogami, presented at the 90th Annual Meeting of Pharmaceutical Society of Japan, Sapporo, July, 1970, No. OB10-1.

Table I. Adsorbed Amount at Equilibrium Concentration 2×10^{-3}M (M_c) and Thermodynamic Functions of Adsorption (ΔG, ΔH, and ΔS) of Benzoic Acid Derivatives

No.	Adsorbates	$M_c\times10^{4}$ a) (mole/g)	ΔG a) (kcal/mole)	ΔH (kcal/mole)	ΔS (*e.u.*)
1	benzoic acid	2.07	0.921	−8.00	−28.78
2	*o*-nitrobenzoic acid	1.67	1.027	−4.01	−16.26
3	*m*-nitrobenzoic acid	4.96	0.000	−2.47	− 7.97
4	*p*-nitrobenzoic acid	4.91	0.152	−3.97	−10.17
5	*o*-aminobenzoic acid	4.20	0.451	−2.67	−10.07
6	*m*-aminobenzoic acid	2.97	0.643	−1.19	− 5.91
7	*p*-aminobenzoic acid	3.41	0.454	—	—
8	*o*-methoxybenzoic acid	1.98	1.248	−7.55	−28.38
9	*p*-methoxybenzoic acid	4.24	0.319	−2.57	− 9.35
10	*o*-methylbenzoic acid	2.01	1.009	−4.61	−18.12
11	*m*-methylbenzoic acid	3.67	0.655	−3.60	−13.73
12	*p*-methylbenzoic acid	3.61	0.626	−2.45	− 9.92
13	*o*-chlorobenzoic acid	1.74	1.169	−3.17	−14.00
14	*m*-chlorobenzoic acid	4.45	0.535	−1.13	− 5.37
15	*p*-chlorobenzoic acid	4.37	0.414	−2.39	− 9.05
16	*o*-bromobenzoic acid	1.94	0.848	−3.91	−14.77
17	*m*-bromobenzoic acid	5.14	0.132	−2.65	− 8.97
18	*p*-bromobenzoic acid	5.05	0.224	−3.06	−10.59
19	*o*-hydroxybenzoic acid	3.32	0.902	—	—
20	*m*-hydroxybenzoic acid	2.66	0.971	—	—
21	*p*-hydroxybenzoic acid	2.95	0.841	—	—
22	2,4-dichlorobenzoic acid	3.39	0.599	—	—

a) at 37°

substrate is the most important factor determining its plant growth activity.[18] Although the low adsorbed amount of *ortho*-substituted benzoic acids in the present study corresponds to the characteristic low affinity to hydrophobic moiety, this may have relation to the characteristic high affinity of the groups containing COO^- and *ortho*-substituent to a hydrophilic moiety or such a biological active site as mentioned above.

The fact that the results for *o*-amino- and *o*-hydroxybenzoic acids were not similar to the other *ortho*-substituted ones is reasonably explained by considering that former the compounds have a great tendency to intramolecular hydrogen bonding.

Adsorption of Benzoic Acid Derivatives with Regard to pK_a and Partition Coefficient

Plotting pK_a against the adsorbed amount M_c of *ortho*-, *meta*- and *para*-substituted benzoic acids, there was found no correlation, as shown in Fig. 4. This result was similar to that reported by Moriguchi, *et al.* as to the adsorption of *meta*- and *para*-substituted ones on graphite.[12]

It was easily found that there was no correlation between the adsorption data and the partition coefficient. On comparing the adsorption data with the partition coefficicient presented by Hansh and Fujita,[8] the data should be obtained under the condition that benzoic acid derivatives are in undissociated state, though Moriguchi, *et al.* showed there was no correlation between the adsorption of these compounds in dissociated state on graphite and the partition coefficient. Benzoic acid derivatives in dissociated state, as also in the present study, have a good affinity to water and thus it was reasonably understood that the adsorbability on graphite or carbon black had no correlation to the partition coefficient.

18) K. Fukui, C. Nagata, and T. Yonezawa, *J. Am. Chem. Soc.*, **80**, 2267 (1958).

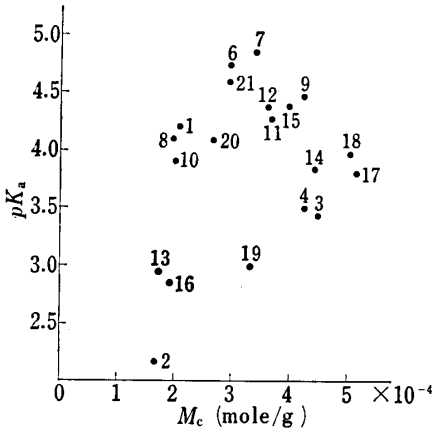

Fig. 4. Plots of pK_a against the Adsorbed Amount at Equilibrium Concentration 2×10^{-3}M, M_c, of Benzoic Acids by Carbon Black at 37° represented by the Same No. as in Table I

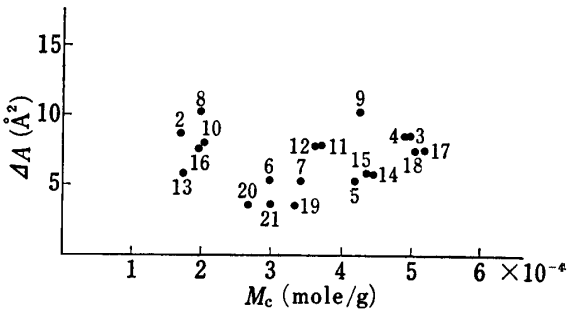

Fig. 5. Plot of the Approximate Plain Size Difference, ΔA,[12] against the Adsorbed Amount at Equilibrium Concentration 2×10^{-3}MM_c, of Benzoic Acid Derivatives by Carbon Black at 37° represented by the Same No. as in Table I

Adsorption of Benzoic Acid Derivatives with Regard to Plain Size of Molecule

Calculating the approximate plain size difference, ΔA, between substituted and unsubstituted benzoic acid derivatives, Moriguchi, *et al.* reported that log *ab* of the adsorption of *meta*- and *para*-substituted compounds on graphite was correlated with ΔA, and concluded that benzoic acid derivatives, or more generally carboxylic acid derivatives, are adsorbed from aqueous solutions dominantly by plain-to-plain stacking on the surface of graphite, and the affinity depends mainly on the plain size of the adsorbed molecule.[12] In the present study containing *ortho*-substituted compounds, plotting the adsorbed amount M_c against ΔA, there was no correlation between both of them, as shown in Fig. 5.

Although it is known that *ortho*-substituents have disturbing effect on the resonance between benzene ring and carboxyl group, COO^- of *ortho*-substituted benzoic acid in the present experimental condition (pH 7, 4) seems to be hydrated in the similar way to that of *meta*- or *para*-substituted one without taking a special behavior. Therefore, the above notion of plain-to-plain stacking concluded by Moriguchi, *et al.* from the adsorption data for *meta*- and *para*-substituted benzoic acids might be questionable. Additionally, the fact that M_c for 2,4-dichlorobenzoic acid was less than that for *p*-chlorobenzoic acid may demonstrate the adsorbability has no relation to ΔA.

Adsorption of Benzoic Acid Derivatives with regard to the Complex Formation with Caffein

Sekiguchi assumed that the solubilizing action of benzoates on caffein is not due to the formation of a complex of established composition but due to the formation of a hydrogen bond between caffein and benzoate, with the water molecule of the solvent acting as the hydrogen-bond donor.[9] On the other hand, Moriguchi, *et al.* obtained a good relationship between pK_a and the free energy change ($-\Delta G$) of complex formation with caffein for benzoates, except for a number of benzoates which have hydrophobic substituents at the *ortho* position, and thus described that direct electrostatic forces between the carboxyl group in the benzoate molecules and the nitrogens of 7-position in the caffein molecule play a dominant role in the complex formation.[10] However, since all the benzoates seem to be in dissociate state in the above experimental condition (pH 7.0) and the electrostatic force concerning *ortho*-substituted benzoate not may be remarkably small compared with that concerning *meta*- or *para*-substituted one, it is questionable why *ortho*-substituted benzoates were exceptional to the linear relationship between pK_a and $-\Delta G$.

Then, plotting the data by Sekiguchi[9] and by Moriguchi, *et al.*[11] against *Mc* in Fig. 6 and 7, respectively, the correlations were obtained without excepting for *ortho*-substituted benzoates.

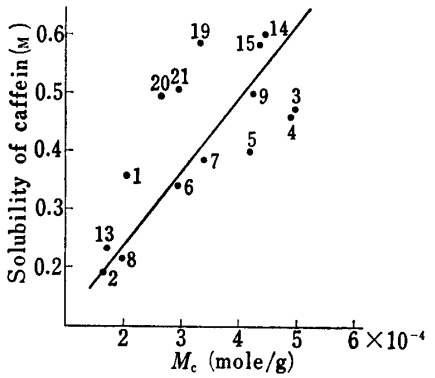

Fig. 6. Plot of the Solubility of Caffein in the Solution Containing 0.4M Benzoic Acid Derivatives at 25°[9] against the Adsorbed Amount at Equilibrium Concentration 2×10^{-3}M, M_c, of the Respective Derivatives by Carbon Black at 37° represented by the Same No. as in Table I

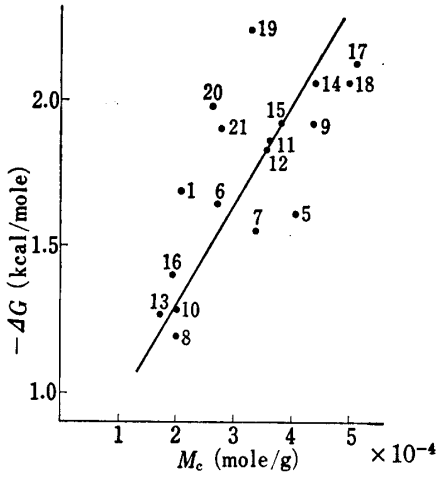

Fig. 7. Plot of the Free Energy Change of Complex Formation with Caffein at 30°, ΔG,[10] against the Adsorbed Amount at Equilibrium Concentration, M_c, by Carbon Black at 37° of Benzoic Acid Derivatives represented by the Same No. as in Table I

Therefore, it seems possible that the hydrophobic interaction also participates in the complex formation between caffein and benzoates. In other words, the interaction forces in the complex formation may be formed of the electrostatic force between COO^- in hydrated state and caffein which is not remarkably variable if benzoates are in dissociated state, and also formed of the force originated by the hydrophobic interaction which is variable upon the substitution of benzoates.

Adsorption of Benzoic Acid Derivatives with Regard to the Binding to Bovine Serum Albumin

Moriguchi observed a linear relationship between logarithm of the binding constant with bovine serum albumin and pK_a for benzoic acid derivatives except for *ortho*-substituted ones.[11]

Plotting the above data by Moriguchi against the adsorbed amount M_c in Fig. 8, the correlation was found without excepting for *ortho*-substituted benzoates. Therefore, the protein binding was considered to take place on the basis of both the electrostatic and the hydrophobic interactions, as was discussed already for the complex formation between caffein and benzoates. Fig. 8 does not contain the data for aminobenzoic acids which deviated remarkably to the lower part. This deviation may be due to the complication of factors affecting the protein binding, because there was found no abnormality in adsorption of aminobenzoic acids by carbon black.

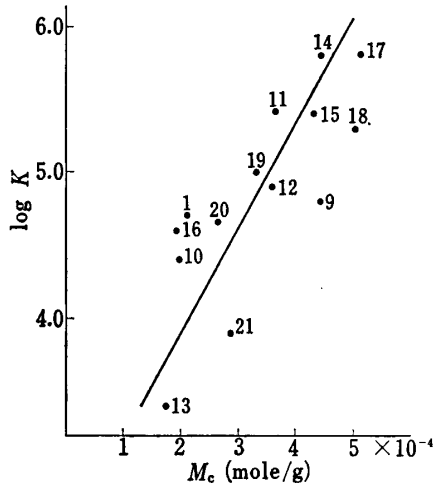

Fig. 8. Plot of Logarithm of Binding Constant to Bovine Serum Albumin at 37°, log K,[11] againt the Adsorbed Amount at Equilibrium Concentration 2×10^{-3}M, M_c, by Carbon Black of Benzoic Acid Derivatives represented by the Same No. as in Table I

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