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Relationship between Competitive Adsorption by Carbon Black from Aqueous Solution and Formation of Molecular Compounds in the Solid Phase for Aminopyrine and Barbituric Acid Derivatives[1,2]

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As an approach to an understanding of the combined effects of drugs, the competitive adsorption of aminopyrine (AM) and barbituric acid derivatives (BA) by carbon black (CB) from aqueous solution was investigated in relation to the formation of molecular compounds between AM and BA.

The formation of molecular compounds of AM with various BA compounds was confirmed by differential scanning calorimetry (DSC), powder X-ray diffractometry and infrared absorption spectrometry.

All adsorption isotherms of AM and BA were in good accordance with Langmuir's equation. From the results of perturbation experiments (20° to 40° transition), it was considered that the mechanisms of adsorption of AM and BA by CB were "chemisorption" and "physical adsorption," respectively.

In competitive adsorption, the decrease of BA adsorbed was larger for compounds that formed molecular compounds with AM than for those that did not. It was considered that adsorption was influenced by an interaction between AM and BA molecules at the surface of CB; the greatest effect in competitive adsorption was seen with AM and barbital, which also formed a molecular compound most readily.

Keywords——pyrabital; aminopyrine; barbituric acid derivatives; molecular compound; competitive adsorption; differential scanning calorimetry; powder X-ray diffractometry; infrared absorption spectrometry

Pyrabital, a molecular compound of aminopyrine (AM) and barbital, has been widely used clinically as an analgesic, and a pharmacological potentiation of the compounds has been demonstrated by many workers. It was reported that the simultaneous intramuscular administration of AM and barbital resulted in a high plasma level compared with single administration of the components.[4,5] It was also reported that the initial plasma concentration of AM increased significantly on simultaneous oral administration with barbital in rabbits as compared with single administration of AM.[6–8] Furthermore, barbituric acid derivatives (BA) such as phenobarbital,[9–11] amobarbital,[12] and hexobarbital[13] have been reported to affect the metabolism of AM significantly.

1) This paper forms Part XV of "Pharmaceutical Interaction in Dosage Forms and Processing." The preceding paper, Part XIV: K. Takayama, N. Nambu, and T. Nagai, *Chem. Pharm. Bull.*, **27**, 715 (1979).
2) A part of this work was presented at the 95th Annual Meeting of the Pharmaceutical Society of Japan, Nishinomiya, April 1975.
3) Location: *Ebara-2-4-41, Shinagawa-ku, Tokyo 142, Japan.*
4) K. Ohata, *Nippon Yakurigaku Zasshi*, **53**, 542 (1957).
5) K. Ohata, *Yakugaku Zasshi*, **78**, 312 (1958).
6) S. Goto, O. Tsuzuki, and S. Iguchi, *Chem. Pharm. Bull.*, **19**, 944 (1971).
7) S. Goto, O. Tsuzuki, and S. Iguchi, *J. Pharm. Sci.*, **61**, 945 (1972).
8) O. Tsuzuki, A. Noda, and S. Iguchi, *Chem. Pharm. Bull.*, **22**, 2459 (1974).
9) A.G. Hildebrandt, I. Roots, M. Speck, K. Saalfrank, and H. Kewitz, *Eur. J. Clin. Pharmacol.*, **8**, 327 (1975).
10) I. Roots, K. Saalfrank, and A.G. Hildebrandt, *Adv. Exp. Med. Biol.*, **1975**, p. 485.
11) H. Niwa and N. Hikichi, *Yakuzaigaku*, **35**, 89 (1975).
12) H. Niwa and H. Sasaki, *Tohoku Yakka Daigaku Kenkyu Nenpo*, **22**, 57 (1975).
13) I. Gut and B.A. Becker, *Arch. Toxicol.*, **34**, 61 (1975).

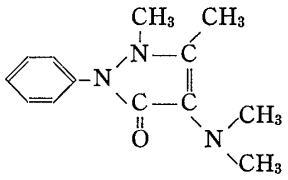
On the other hand, it was shown in previous papers[14–16] that the adsorption of drugs on hydrophobic surfaces such as carbon black (CB) might be related to the uptake process of drugs on the gut wall.

Based on the above considerations, the present study was planned to investigate the competitive adsorption of AM and BA in relation to molecular compound formation as an approach to an understanding of the combined effects of the drugs.

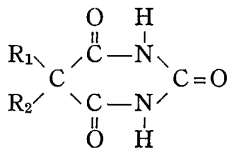
Experimental

Materials——Carbon black (CB), which was the same as that used in the previous work[14] (marketed as "Seisei Shirasagi" by Takeda Chemical Ind., Ltd.) was used after activation by heating. The specific surface area of CB determined by nitrogen gas adsorption was 1250 m^2/g. Aminopyrine (AM) and barbituric acid derivatives (BA) used are listed in Table I. Cyclobarbital calcium was converted to the free base by adding hydrochloride solution, after recrystallization several times from aqueous solution. The purified free bases of BA were used as supplied.

Table I. Aminopyrine and Barbituric Acid Derivatives used in This Study



aminopyrine[a]



barbituric acid derivatives

Barbituric acid derivatives	R_1	R_2
Barbital[b]	Ethyl	Ethyl
Phenobarbital[b]	Ethyl	Phenyl
Cyclobarbital[c]	Ethyl	Cyclohexyl
Pentobarbital[e]	Ethyl	1-Methyl butyl
Amobarbital[d]	Ethyl	Isoamyl
Allobarbital[f]	Allyl	Allyl
Secobarbital[b]	Allyl	1-Methyl butyl

a) Supplied by Sumitomo Chemical Co., Ltd.
b) Supplied by Grelan Pharmaceutical Co., Ltd.
c) Supplied by Shionogi Pharmaceutical Co., Ltd.
d) Supplied by Nippon Shinyaku Co., Ltd.
e) Supplied by Tanabe Seiyaku Co., Ltd.
f) Supplied by Iwaki Seiyaku, Ltd.

Batch Procedure for Determination of the Amount adsorbed——The adsorption procedures were as described in the previous paper,[14] except that 10 mg of CB was added to 10 ml of a 1/30 M phosphate buffer solution (pH 7.0) of each drug. For the study of the competitive adsorption of AM and BA, equimolar amounts of the drug were added in 10 ml of the buffer solution.

Determination of Barbituric Acid Derivatives——After diluting samples with the buffer solution used for the adsorption experiment, the concentration of the drug was determined by measuring the absorbance at 240 nm, using the ultraviolet (UV) absorption method with a Hitachi 323 spectrophotometer. When AM was also present, the method developed by Ohata[4] was used.

Determination of Aminopyrine——The colorimetric method developed by Ono[17] was used. The determination was not influenced by the presence of BA.

Preparation of Aminopyrine-Barbituric Acid Derivative Coprecipitates——Equimolar amounts of AM and BA were dissolved in water or ethanol and recrystallized in the usual way. The coprecipitate formed was dried under reduced pressure and stored in a desiccator until required.

14) H. Nogami, T. Nagai, E. Fukuoka, and H. Uchida, *Chem. Pharm. Bull.*, **16**, 2248 (1968).
15) H. Nogami, T. Nagai, and H. Uchida, *Chem. Pharm. Bull.*, **17**, 168 (1969).
16) H. Nogami, T. Nagai, and H. Uchida, *Chem. Pharm. Bull.*, **17**, 176 (1969).
17) S. Ono, R. Onishi, M. Tange, K. Kawamura, and T. Imai, *Yakugaku Zasshi*, **85**, 245 (1965).

Preparation of Aminopyrine-Barbituric Acid Derivative Fused Mixtures——A physical mixture of equimolar AM and BA mixed thoroughly in an agate morter was heated to complete fusion in a melting point measuring apparatus. The melt was allowed to solidify and stored in a desiccator until required.

Differential Scanning Calorimetry (DSC)——DSC was carried out using a Perkin-Elmer model 1B differential scanning calorimeter.

Powder X-Ray Diffraction Studies——Powder X-ray diffraction patterns were obtained with a Rigaku Denki diffractometer using Ni-filtered Cu-Kα radiation.

Infrared (IR) Absorption Spectrum——IR spectra were measured with a Shimadzu IR 400 spectrophotometer by the KBr disk method.

Results and Discussion

Confirmation of Molecular Compound Formation by Aminopyrine and Barbituric Acid Derivatives in the Solid Phase

Molecular compound formation by AM and BA was confirmed by the DSC curves, powder X-ray diffraction patterns and IR absorption spectra. As an example, DSC curves of a physical mixture and a fused mixture of AM and amobarbital are shown in Fig. 1. It was reported[18] that the formation of a molecular compound in the solid phase is indicated by both endothermic and exothermic peaks, as shown in Fig. 1. The fused mixture can be regarded as the molecular compound. A similar phenomenon was observed with pentobarbital.

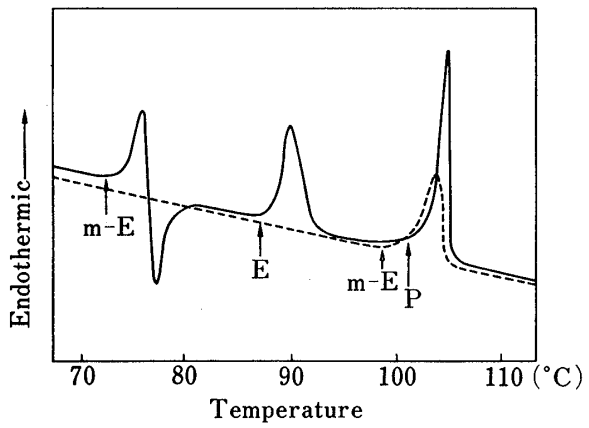


Fig. 1. DSC Curves for the Fused Mixture and the Corresponding Physical Mixture of Aminopyrine and Amobarbital at a Scanning Speed of 2°/min

—— : physical mixture,
····· : fused mixture.
m-E : metastable melting point,
E : normal eutectic point,
P : peritectic point.

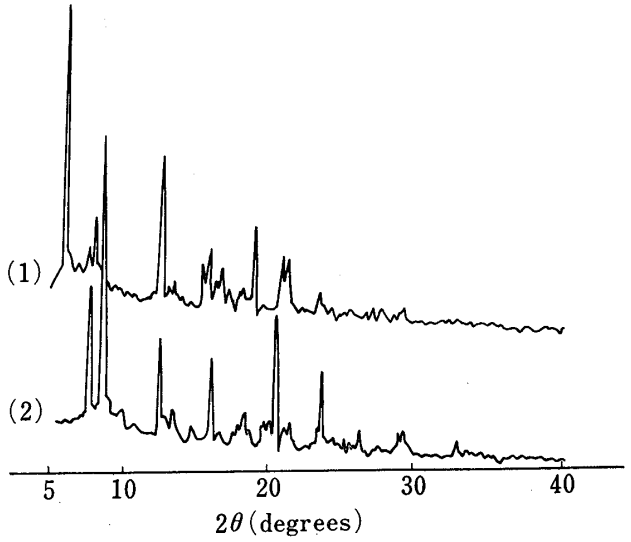


Fig. 2. Powder X-Ray Diffraction Patterns of the Coprecipitate and the Corresponding Physical Mixture of Aminopyrine and Amobarbital (Cu-Kα Radiation)

(1) coprecipitate,
(2) physical mixture.

To confirm the formation of the molecular compound, the powder X-ray diffraction patterns of AM–BA coprecipitates were compared with those of their physical mixtures. As an example, the diffraction patterns of the coprecipitate and the corresponding physical mixture of AM and amobarbital are shown in Fig. 2. The patterns are different, indicating a difference in crystalline form between the mixture and coprecipitate. The coprecipitate can be regarded as the molecular compound. A similar phenomenon was observed with pentobarbital.

18) K. Sekiguchi, I. Himuro, I. Horikoshi, T. Tsukada, T. Okamoto, and T. Yotsuyanagi, *Chem. Pharm. Bull.*, 17, 191 (1969).

Further confirmation of the formation of the molecular compound was made by comparing the IR absorption spectra of the physical mixture and the coprecipitate of AM and BA. In the coprecipitate, the absorption band between 1650 cm^{-1} and 1750 cm^{-1} due to C=O groups of AM and BA and that between 2800 cm^{-1} and 3200 cm^{-1} due to the NH group of BA were substantially changed, in accord with the report of Sohár *et al.*[19] The coprecipitate can be regarded as the molecular compound. Thus, it was concluded that amobarbital and pentobarbital formed molecular compounds with AM in the same way as barbital, pentobarbital and cyclobarbital, which have already been reported.[18]

Mechanism of Adsorption of Aminopyrine and Barbital by Carbon Black from Aqueous Solution

The adsorption isotherms of AM and barbital by CB at 20°, 30° and 40° are well described by the following Langmuir equation (1), as shown in Fig. 3, for example.

$$M = \frac{abC}{1+bC} \tag{1}$$

Here M is the amount adsorbed at concentration C in solution, a is the amount adsorbed when the entire surface is covered by a monolayer, and b is the equilibrium constant of the adsorption process. Equation (1) can be rearranged as follows:

$$\frac{C}{M} = \frac{1}{ab} = \frac{C}{a} \tag{2}$$

Perturbation experiments on the adsorption of AM and barbital by CB are shown in Fig. 4. By changing the temperature in the range of 20° to 40°, as shown in Fig. 3 and 4, it was found that the adsorption was irreversible and that the amount adsorbed increased with rise of temperature for AM. On the other hand, the adsorption of barbital was reversible and the amount adsorbed decreased with rise of temperature. Thus, the mechanisms of the adsorption of AM and barbital by CB are of "chemisorption" type and "physical adsorption" type, respectively. Similar results were reported for guanethidine sulfate and hydrochlorothiazide.[20]

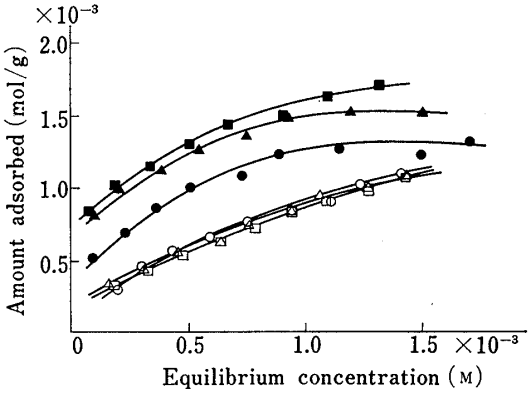


Fig. 3. Adsorption Isotherms of Aminopyrine and Barbital on Carbon Black from Aqueous Solution at Various Temperatures

20° 30° 40°
● ▲ ■ aminopyrine
○ △ □ barbital

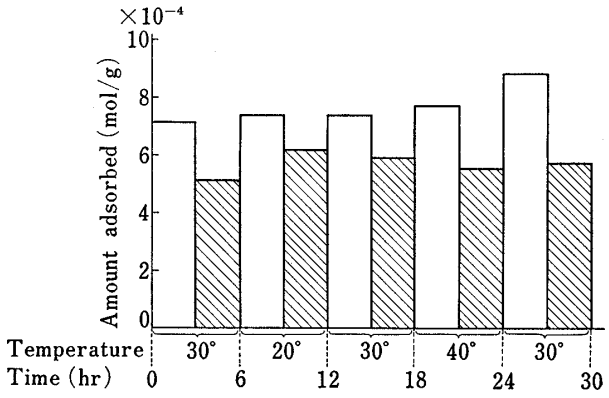


Fig. 4. Perturbation Experiments on the Adsorption of Aminopyrine and Barbital by Carbon Black from Aqueous Solution at an Initial Concentration of 1.00×10^{-3} M

□ aminopyrine; ▨ barbital.

19) P. Sohár, E. Orbán, and G. Tóth, *Acta Chim. Acad. Sci. Hung.*, **55**, 87 (1968).
20) H. Ueda and T. Nagai, *Chem. Pharm. Bull.*, **26**, 1353 (1978).

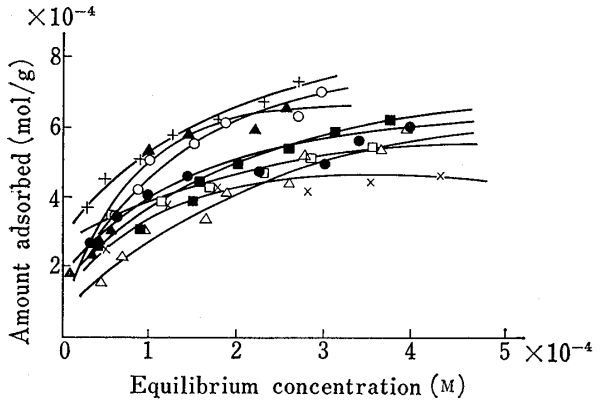


Fig. 5. Adsorption Isotherms of Aminopyrine on Carbon Black from Aqueous Solution in the Presence of Various Barbituric Acid Derivatives at 40°

+ : aminopyrine alone.
○ : barbital,
△ : phenobarbital,
□ : cyclobarbital,
● : pentobarbital,
▲ : amobarbital,
■ : allobarbital,
× : secobarbital.

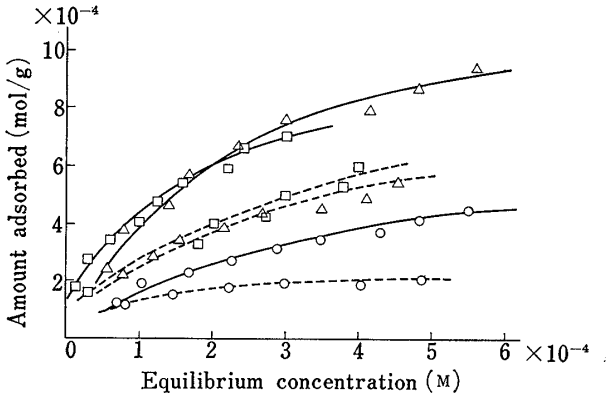


Fig. 6. Adsorption Isotherms of Barbituric Acid Derivatives on Carbon Black from Aqueous Solution in the Presence of Aminopyrine at 40°

—— : barbituric acid derivatives alone,
····· : in the presence of aminopyrine,
○ : barbital,
△ : phenobarbital,
□ : cyclobarbital.

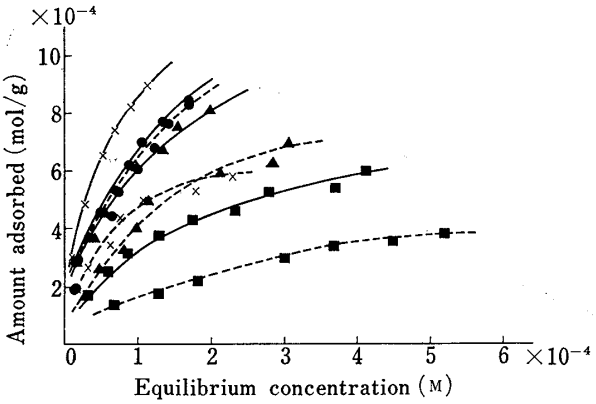


Fig. 7. Adsorption Isotherms of Barbituric Acid Derivatives on Carbon Black from Aqueous Solution in the Presence of Aminopyrine at 40°

—— : barbituric acid derivatives alone,
····· : in the presence of aminopyrine,
● : pentobarbital,
▲ : amobarbital,
■ : allobarbital,
× : secobarbital.

Competitive Adsorption of Aminopyrine and Barbituric Acid Derivatives by Carbon Black from Aqueous Solution

The adsorption isotherms for AM coexisting with BA by CB from aqueous solution at 40° are shown in Fig. 5, and those for BA coexisting with AM are shown in Fig. 6 and 7. As can be seen in Fig. 5, 6 and 7, all the adsorption isotherms were in good accordance with Langmuir's equation and the Langmuir constants a and b were calculated. The values obtained are shown in Tables II and III. Table II shows the values for AM coexisting with BA and Table III shows those for BA coexisting with AM.

The order of amount adsorbed of BA without AM was in good accordance with that given in the previous paper.[15] As shown in Fig. 5, 6, and 7, the amount adsorbed of AM and BA usually decreased in a competitive fashion. The values of D% in Table II were in the range of 42 to 63%, while those in Table III were scattered. As for the b values, an increase was observed for AM in competitive adsorption, but not for BA. This phenomenon appears to correspond to the difference in the mechanism of adsorption of the drugs, as mentioned above.

Further investigations are required to permit a detailed discussion of the mechanism of competitive adsorption, but the scattered values of a and b in Tables II and III indicate that the competitive adsorption of BA and AM occurs at heterogeneous adsorption sites on CB.

Table II. Competitive Adsorption of Aminopyrine and Barbituric Acid Derivatives on Carbon Black from Aqueous Solution at 40°

	$a\times10^{4}$ a)	$b\times10^{-4}$ b)	$\Delta a\times10^{-4}$ c)	D% d)	$\Delta b\times10^{-4}$ c)
Aminopyrine alone	17.794	0.458	——	——	——
Aminopyrine with					
Barbital	8.865	1.111	8.929	50.18	−0.653
Phenobarbital	7.849	4.719	9.945	55.89	−4.261
Cyclobarbital	6.693	1.256	11.10	62.39	−0.798
Pentobarbital	6.557	1.584	11.24	63.15	−1.126
Amobarbital	7.651	11.47	10.14	57.00	−11.01
Allobarbital	8.905	1.591	8.889	49.96	−1.133
Secobarbital	10.40	0.495	7.399	41.58	−0.037

a) Langmuir's constant a of adsorption of aminopyrine (mol/g).
b) Langmuir's constant b of adsorption of aminopyrine (1/mol).
c) Δa and Δb are the differences from the a and b values of aminopyrine alone, respectively.
d) Percent ratio of Δa to a value for aminopyrine alone.

Table III. Competitive Adsorption of Barbituric Acid Derivatives and Aminopyrine on Carbon Black from Aqueous Solution at 40°

Barbituric acid derivative	without aminopyrine		with aminopyriue		$\Delta a\times10^{4}$ c)	D% d)	$\Delta b\times10^{-4}$ c)
	$a\times10^{4}$ a)	$b\times10^{-4}$ b)	$a\times10^{4}$ a)	$b\times10^{-4}$ b)			
Barbital	7.199	0.274	2.382	1.384	4.817	66.91	−1.110
Phenobarbital	12.89	0.437	6.662	0.701	6.225	48.30	−0.264
Cyclobarbital	8.591	1.151	7.669	0.585	0.922	10.73	0.566
Pentobarbital	12.63	1.109	9.337	2.606	3.289	26.05	−1.497
Amobarbital	10.87	1.347	8.251	0.811	2.619	24.09	0.536
Allobarbital	7.174	0.887	6.662	0.389	0.512	7.14	0.498
Secobarbital	12.76	1.985	10.70	0.740	2.060	16.15	1.245

a) Langmuir's constant a of adsorption of barbituric acid derivatives (mol/g).
b) Langmuir's constant b of adsorption of barbituric acid derivatives (1/mol).
c) Δa and Δb are the differences of the a and b values without aminopyrine and with aminopyrine, respectively.
d) Percent ratio of Δa to a value without aminopyrine.

Competitive Adsorption of Aminopyrine and Barbituric Acid Derivatives in Relation to Formation of the Molecular Compounds

Tables II and III show values of D%, which are percent ratios of Δa to the values of AM and BA alone. $(D\%)_{Total}$, which are the sums of D% given in Tables II and III for each BA, are shown in Table IV. $(D\%)_{Total}$ values can be classified into three groups. Barbital and phenobarbital, which form molecular compounds with AM easily, shows large

Table IV. $(D\%)_{Total}$ Values for Barbituric Acid Derivatives[a)]

Barbituric acid derivative	$(D\%)_{Total}$
Barbital	117.09
Phenobarbital	104.19
Cyclobarbital	73.12
Pentobarbital	89.20
Amobarbital	81.09
Allobarbital	57.73
Secobarbital	57.10

a) $(D\%)_{Total}$ is the sum of the D% values given in Tables II and III for each barbituric acid derivative.

$(D\%)_{Total}$ values of almost 110. Cyclobarbital, pentobarbital and amobarbital, with $(D\%)_{Total}$ values of about 80 (73—89), form molecular compounds with AM, but not as easily as barbital and phenobarbital. $(D\%)_{Total}$ values for allobarbital and secobarbital are about 57, and this may be related to their failure to form molecular compounds with AM.

Based on these results, it is considered that the adsorption of AM and BA by CB is influenced by an interaction between the drug molecules, and that the main effect of barbital on the adsorption of AM, as reflected in the values of $(D\%)_{Total}$ in competitive adsorption, might be related to the remarkable ease of formation of the molecular compound of AM and barbital. The reason for this is not clear, but may be related to the chemical structure of BA. BA has an ethyl group at R_1, whereas allobarbital and secobarbital, which do not form molecular compounds with AM, have an allyl group at R_1. The chemical structure also affects the adsorbability, that is, the conformation or orientation of drug molecules at the hydrophobic CB surface on adsorption from aqueous solution. If more detailed biopharmaceutical data could be obtained, this kind of adsorption study might give valuable insights into the clinical effects of the combined administration of drugs.

Acknowledgement The authors gratefully acknowledge generous supplies of chemicals from Grelan Pharmaceutical Co., Ltd., Shionogi Pharmaceutical Co., Ltd., Nippon Shinyaku Co., Ltd., Tanabe Seiyaku Co., Ltd., Sumitomo Chemical Co., Ltd., and Iwaki Seiyaku Ltd. Thanks are also due to Mr. Masao Iijima for his assistance in the experimental work.