Interaction of Pharmaceuticals with Beta-cyclodextrin Ⅱ

Interaction with Barbituric and thiobarbituric Acid

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Beta-cyclodextrin과 醫藥品과의 相互反應Ⅱ. Barbituric acid및 Thiobarbituric acid 誘導體와 Beta-cyclodextrine과의 相互反應

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Barbituric acid 및 thiobarbituric acid 유도체와 beta-cyclod-extrin과의 相互反應을 水溶液과 有機溶媒中에서 各 藥物을 溶解度, 分子容, 熱力學的인 面에서 分析 檢討하였다.

本實驗에 사용한 藥物이 모두 溶液에서는 beta-cyclodextrin과 反應하여 溶解度가 增加하였으며 固型의 침전을 形成하지는 않았으나 比較的 安定한 會合이 이루어졌다. 本反應의 機轉은 主로 beta-cyclodextrin의 主要한 性質의 하나인 inclusion能에 依하여 이루어졌음을 報告한다.

The conception of "inclusion compound" or "clathrate" was defined by W. Schlen Jr[1]. to describe the positional relationship of two components which form certain types of association between host and gust molecule. The inclusion formation phenomenon has been utillzed in various applications.

(1) Sepaqation and purification of compounds [2,3]

(2) Stabilization of drugs [4][5]

(3) Solubilization of nearly insoluble drugs [6,7]

(4) Separation of optically active compounds [8-13]

Since the discovery of the cyclodextrins or schardinger dextrins considerable attention has been paid to them as characteristic inclusion formation host molecule. As a result of their cyclic structure and internal cavities, the cyclodextrins have been of considerable

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interest because of distinctive ability to form inclusion compounds with a wide varity of compounds. It is well known that usually several host molecules are needed to provide the encasing structure, but in most cases one molecule of cyclodextrin offers the geometry necessary for inclusion.

In the early part of this study[14] the interactions of beta-cyclodextrin with a series of sulfonamides was studied in aqueous solution. In this experiments a solubility method emloying organic solvents to minimize any interaction due to hydrogen bonding or polar effects was also investigated.

It was the objective of this report to investigate and compare the interaction tendencies of various agents with beta-cyclodextrin in detail characterizing the thermodynamic behaviours of the interaction and correlating them with physical factors, such as solubility, molecular volume.

Barbituric and thiobarbituric acid derivatives were selected for this study.

EXPERIMENTAL

Materials and reagents:——Beta-cyclodextrin $[\alpha]_D^{25} = 162$ (c = 1, in water) barbital; m. p. 190-191°; amobarbital, m. p. 156-157°; cyclobarbital, m. p. 173°; allobarbital, m. p. 170-171°; Hexobarbital, m. p. 147-148°; pheonbarbital, m. p. 176-178; secobarbarbital, m. p. 98°; thiopental, m. p. 157; mephobarbital, m. p. 177.

Carbon tetrachloride.

Apparatus:——A constant temperature incubator, set at 20±0. 5°, 30±0. 5°, 37±0. 5°, with magnetic stirrer. 100 ml. capacity vials with gum rubber stoppers, Beckman DU spectrophotometer,

Procedure

Preparation of the beta-cyclodextrin:——The beta-cyclodextrin was prepared by the procedure used in the previous report[14].

Solubility studies:——The solubility method of Higuchi and Lach[15] was used to study compliex formation. The experimental procedures were similar to those outlined in the previous paper of this series. Excess quantities of the substances to be studied were accurately weighed into 100 ml capacity vials together with varing concentrations of beta-cyclodextrin. To each vial 50 ml distilled water or carbontetrachloride then was added, after which they were capped with rubber stopper. The vials were then stirred with magnetic stirrer in a constant temperature incubator set at 20°, 30°, and 37° for 24 hours, sufficient time for the equilibrium.

Equilibria were approached from higher as well as lower temperatures within 24 hours. All samples were run in triplicate.

Method of analysis——After equilibrium has been eached, aliquot portions of the supernatant liquid were removed and if undissolved materials were contained in solution at the end of equilibriation, cotton were used on the pipet tips.

The concentrations of barbituric and thiobarbitunric acid derivatives were determined at 240 mμ and 305 mμ, respectively, according to UV absorption method after diluting with alkaline borate buffer pH 9.6, using a Beckman DU spectrophotometer. When carbontetrachloride was used as solvent, a suitable quantities of supernatant solution were pipetted into mess-flask and evaporating the carbontetrachloride in the water bath at 40° and then diluted with alkaline borate buffer pH 9.6.

RESULTS AND DISCUSSION

Solubility methods in aqueous and non-aqueous solutions were tested for this inclusion complexation study. The use of carbontetrachloride as a nonaqueous solution to minimize any interaction due to hydrogen bonding or polar essects resulted in no increase of barbituric and thiobarbituric acid derivatives solubilities in the presence of beta-cyclodextrin. This lac of interaction of tested compounds with beta-cyclodextrin in such organzeid solvent indicate that (water) must play a major factor in interactions of this tÁpe in solution[5].

The solubility method employing an aqueous solution was, therefore, used to study these interactions.

As described in the previous report of sulfonamides and beta-cyclodextrin interaction[14], definite interaction were also observed with all of the compounds employed in this experiments.

The relationships between the equilibrium solubilities of the various barbituric and thiobarbituric compounds and beta-cyclodextrin concentration are clearly seen by the linear interaction isotherms.

As seen from the Table I., II. and Fig. 1. and 2, no plateau region indicating formation of insoluble complexes was found. As we know in phase diagrams showing no plateau region, stoichiometries and formation constants can't be calculated precisely, but from the linear interaction istherms formation constants were calculated with the assumption being made that monomolecular reaction took place between these compounds and beta-cyclodextrin.

Table I. Total soubility of barbital in water containing beta-cyclodextrin at 20° and 30° C.

Beta-cyclodextrin added to system	Total barbital at saturation Concn. $\times 10^2$M	
Concn. $\times 10^3$M.	20°C	30°C
0	3.5260	4.4727
0.8818	3.6033	4.5475
1.7636	3.6709	4.6176
2.6454	3.7579	4.6853

3.5272	3.8352	4.7819
4.4090	3.9221	4.8495
5.2908	3.9897	4.9171
6.1726	4.0767	4.9847
7.0544	4.1539	5.0717
7.9362	4.2312	5.1490
8.8138	4.3085	5.2069

Table II. Total solubility of amobarbital in water containing beta-cyclodextrin at 20°, 30° and 37°C

Betacyclodextrin added to system	Total amobarbital at saturation. Concn. $\times 10^3$M.		
Concn. $\times 10^3$M.	20°C	30°C	37°C
0	1.9378	2.8187	3.3903
0.8818	2.5863	3.4871	4.0683
1.7636	3.2062	4.1167	4.6979
2.6454	3.8358	4.7948	5.3276
3.5272	4,5042	5.3276	5.9330
4.4090	5.0370	6.0734	6.6595
5.2908	5.6957	6.7806	7.2891
6.1726	6.1994	7.2649	7.9429
7.0544	6.8774	8.0398	8.5967
7.9362	7.6911	8.7663	9.2022
8.8183	8.2239	9.3184	9.8803

Table III. Total soubility of secobarbital in water containing betacyclodextrin at 20°, 30° and 37°C.

| Betacyclodextrin added to system	Total secobarbital at saturation. Conc. $\times 10^3$M.		
Concn. $\times 10^3$M.	20°C	30°C	37°C
0	3.9590	5.3941	6.7308
0.8818	4.6518	6.1117	7.4231
1.7636	5.4931	6.9035	8.1654
2.6454	6.2849	7.5716	8.9325
3.5272	7.0767	8.4129	9.5511
4.4090	7.8685	9.1057	10.2439
5.2908	8.5861	9.9470	10.9368
6.1726	9.4026	10.6151	11.6296
7.0544	10.1450	11.2832	12.3719
7.9362	10.9862	12.1740	13.0152
8.8183	11.8770	12.7678	13.8071
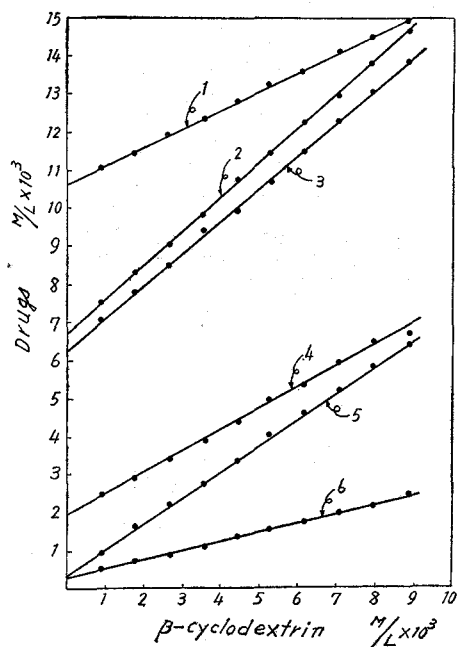
Fig. 1. Influence of beta-cyclodextrin on the solubilities of barbituric and thiobarbituric acid derivatives at 20°C Key: 1, cyclobarbital; 2, phenobarbital; 3, allobacbital, ; 4, thiopental; 5, hexobarbital; 6, mephobarbital.

Fig. 2. Influence of betacyclodextrin on the solubilities of barbituric and thiobarbituric acid derivatives at 30°C. Key: 1, allobarbital; 2, cyclobarbital; 3, phenobarbital; 4, hexobarbital; 5, thiopental; 6, mephobarbital.

The magnitude of relative interaction tendencies was represented by the increase in solubilites of test compounds and the slopes of the interaction isotherms. It is difficult to interpret these data mathematically, however, certain physical and chemical properties of these pharmaceuticals are evient. Fig. 3 shows the correlation of slope with log S_o (S_o: solubilities in the absence of beta-cyclodextrin) as an answer of these ploblems.

The correlation coefficient (-0.7267) indicates that slope is significantly correlated at 0.05 level. It is shown that the more soluble the drugs the more they were interacted with beta-cyclodextrin. This may suggest that the interaction mainly depends on the solubility of comoounds.
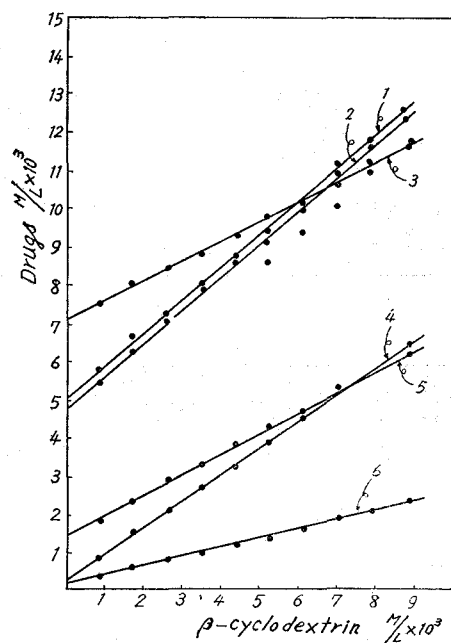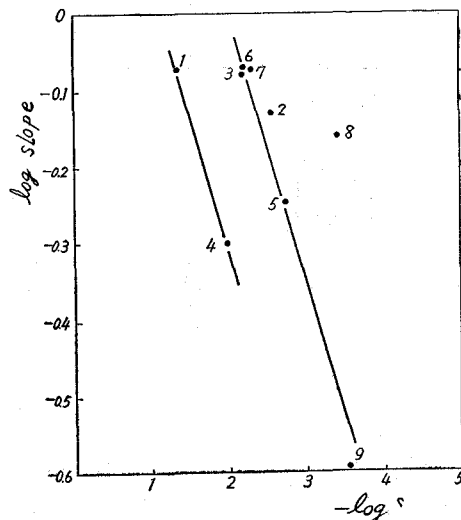
As described above the inclusion forma-

Fig. 3. Relationship of slope of interaction isotherm of drugs with the betacyclodextrin to initial solubility at 30°C Key: 1, barbital; 2, amobarbital; 3, cyclobarbital; 4, allobarbital; 5, hexobarbital; 6, phenobarbital; 7, secobarbtail; 8; thiopental; 9, mephobarbital.

tion between host and guest molecules depends upon the molecular size and the positional relationships of two components. Fig. 4. shows the solubility increase of barbituric and thiobarbituric acid derivatives with varying molecular volumes of them.

If the 5—substituted hydrocarbon chains in these pharmaceuticals may be correlated to the inclusion formation it is assumed that the solubility increase shown in Fig. 4. is the result of the difference of the molecular volumes and the chain length.

Pauli et. al. [16] reported that the relative reactivities of a series of phenyl-substituted carboxylic acids with beta-cyclodextrin mainly depends upon the chain length of carboxylic acids. The increased bulk and rigidity of the chainresulting from the conjugated ethylenic linkages of unsaturated carboxylic acids reduced reactivity with betacyclodextrin mainly depends upon the chain length of carboxylic acids.

The increased bulk and rigidity of the chain resulting from the conjugated ethylenic linkages of unsaturated carboxylic acids reduced reactivity with beta-cyclodextrin, but in saturated carboxylic acids the longer the chain length, the more they were interacted with betacyhlodextrin.

As seen in Fig. 4. no regularity was found in barbituric and thiobarbituric acid derivatives, but amobarbital, cyclobarbital, phenobarbituric acid derivatives, but

Fig. 4. Relationship between solubility incr-ease by beta-cyclodextrin and molecular molecular volume of barbituric and thioba-rbituric acidderives at 30c° Key: 1. banrb-ital; 2, amobarbital, 3, cyclobarbital; 4, allobarbital, 5, hexobarbital; 6. phenobar-bital; 7, secobarbital; 8, thiopental; 9, mephobarbital.

amobarbital, cyclobarbital, phenobarbital, scobarbital and thiopental show same trends and especially barbital show relatively low solubility increase as the results in Fig. 3.

This phenomenon can be attributed to the fact that the shorter chain lengths of barbital and allobarbital would be more rigid than other drugs. The moleccular volume [17] was calculated by summing the respective atomic volumes. For the convenience of discussion the term "solubility increase" is defined as the amount solubilized at the equili-brium concentration $4,409 \times 10^{-3}M$ of beta-cyclodextrin minus the amount solubilized in the absence of beta-cyclodextrin.

The formation constants, free energy change and other thermodynamic values were calculated in a manner analogous to those employed in previous studies[14]. The magnitude of the formation constants and free energy change indicate the existence of extremely stable complexes. The thermodynamic functions of solubilization of barbituric and thiobarbituric acid compounds are shown in Table IV.
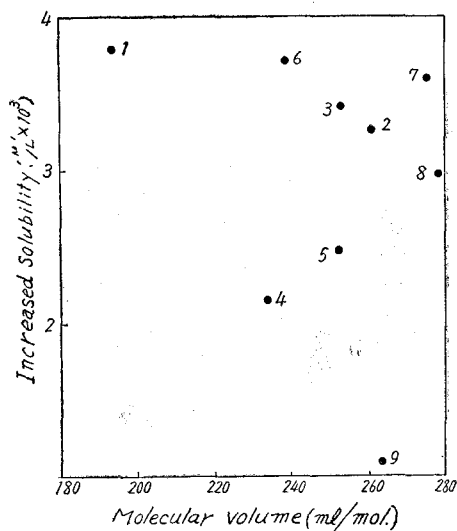
Table IV. Thermodynamic functions of interaction of beta-cyclodextrin and barbituric acid derivatives.

Drugs	Temp. °C	Formation constant, K.	$-\triangle F$ (kcal/mole)	$-\triangle H$ (kcal/mole)	$-\triangle S$ (e. u.)
Barbital	20	217	3.13	10.3	24.4
	30	121	2.89	10.3	24.4
Amobarbital	20	1290	4.17		1.5
	30	1000	4.16	4.6	1.5
	37	849	4.15		1.5
Cyclobarbital	20	1300	4.18		18.8
	30	750	3.99	9.7	18.8
Allobarbital	20	155	2.94		24.1
	30	88	2.70	10.0	24.1
Hexobarbital	20	930	3.98		2.7
	30	710	3.95	4.76	2.7
Phenobarb tal	20	1720	4.34		15.9
	30	1030	4.18	9.0	15.9
Secobarbital	20	1890	4.39		25.3
	30	970	4.14	11.8	25.2
	37	610	3.95		25.3
Thiopental	20	7750	5.22		6.1
	30	5220	5.15	7.0	6.1

The slop of the log K against 1/T was supposed to be constant in the present temperature range as indicated in Fig. 5. From this slopes enthalpy change was calculated.

In an attempt to elucidate further the complex nature of this interaction mechanism, differential spectroscopy was studied with barbital and phenobarbital in beta-cyclodextrin aqueous solution. This results indicate that hydrogen bonding and other bonding effects of drugs and hyclodextrin are nearly indifferent to this interaction. The inclusion formation by beta-cyclodextrin must be a significant factor in this type of interaction.

CONLUSION

The relative interaction tendencies between beta-cyclodextrin and barbituric and thiobarbiaric acid derviatives was
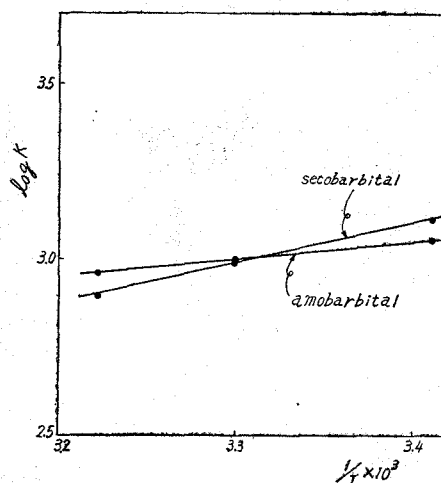

Fig. 5; Relationship between formation constants of amobarbital and secobarbital and temperature.

determined in detail, characterizing the

thermodynamic behaviours of the interaction and correlating them with physical factors, such as solubility, molecular volume. A definite interaction occurred with all compounds. tested.

The large formation constants and free energies of formation suggest a high degree of stability for the complexes and favorable combining conditions between the barbituric and thiobarbituric acid derivatives and beta-cyclodextrin.

The complexes formed are considered to be due to inclusion formation and in part, to other attractive forces existing between the guest and host molecule.

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