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Interaction of Barbituric Acid Derivatives with Chloral Hydrate[1]

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The solubilization of barbiturates by chloral hydrate was investigated by solubility measurement and a molecular interaction was found to be due to the hydrogen bonding from infrared and ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) spectra. In water the stability constants of 1:2 complex, $K_{1:2}$, were larger than those of 1:1 complex, $K_{1:1}$. The stability constants in benzene were larger compared to those in water. For the N-methyl series $K_{1:2}$ values could not be obtained. The stability constants decreased with increasing of the polarity of solvent and pK_a of proton donors. It was found that chloral hydrate had the extraordinary reactivity in molecular interaction. The intensities of the OH strechting bands of chloral hydrate decreased by the addition of phenobarbital and a new band due to the hydrogen bonded OH appeared. The OH protons of chloral hydrate shifted to downfield by the addition of metharbital. Meanwhile, the ^{13}C signals of carbonyl groups at 2- and 4- or 6-C of hexethal shifted to lower field in the presence of chloral hydrate. The former shift occurs to the farthest at the lower concentration of ligand and the latter proceeds with the increase of ligand. This may indicate that 2-carbonyl group forms the hydrogen bond with chloral hydrate preceedingly and 4- or 6-carbonyl group participates subsequently.

The solubilization of barbituric acid derivatives (BA) by various compounds has been studied by many workers, *i.e.* formation of micelle with nonionic surfactants,[3] inclusion complexations with α- and β-cyclodextrins,[4] and formation of hydrogen-bonded complexes with various proton donors.[5] The structural features of BA which are responsible for molecular interaction are CONH groups capable of hydrogen bonding and lipophilic 5-C substituent susceptible to hydrophobic bonding.

In this study using chloral hydrate and the related compounds as the solubilizing agent the solubilization of BA was quantitatively investigated by solubility measurement and the structure of the complexes formed was postulated from infrared (IR), and ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) spectra.

Chloral hydrate is known to be a strong proton donor and its pharmacological activity is co-operative to BA. Then the results of this study may be available to dosage formulation and also may concern with the molecular interaction related with the pharmacological mechanism.

Experimental

Materials——Most of BA were obtained from commercially available sources and recrystallized from ethanol-water mixture. Hexethal was synthesized referring to the literature.[6] Chloral hydrate was obtained commercially and recrystallized from benzene. All other materials were of analytical grade.

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3) A.A. Ismail, M.W. Gouda, and M.M. Motawi, *J. Pharm. Sci.*, **59**, 221 (1970); K. Ikeda, K. Kato, and T. Tukamoto, *Chem. Pharm. Bull.* (Tokyo), **19**, 2510 (1971).
4) A.L. Thakkar, P.B. Kuehn, J.H. Perrin, and W.L. Wilham, *J. Pharm. Sci.*, **61**, 1841 (1972); K. Koizumi, K. Mitsui, and K. Higuchi, *Yakugaku Zasshi*, **94**, 1515 (1974).
5) K.C. Tewari, F.K. Schweighardt, J. Lee, and N.C. Li, *J. Mag. Reson.*, **5**, 238(1971).
6) D.L. Tabern and E.A. Volwiler, *J. Am. Chem. Soc.*, **59**, 1961 (1935).

Solubility Studies——BA in the excess of the solubility was taken in vials which contain solutions of varying concentration of ligands. The vials were closed and shaken for about 30 hr at 25°. After equilibration aqueous solution was pipetted out through cotton filter and then diluted with 0.1M borate buffer (pH 9.0). The concentration of barbiturates was determined spectrophotometrically. For the determination in organic solvent the solution was shaken with borate buffer (pH 9.0) for the extraction of BA and the aqueous phase was subjected to spectrophotometry. Stability constants were calculated from the solubility diagrams according to the methods of Kakemi, *et al.* and Higuchi, *et al.*[7,8]

IR Studies——IR spectra were recorded by a Perkin Elmer model 124 double-beam spectrophotometer using potassium bromide cell whose length is from 0.53 mm to 1.41 mm. Organic solvents were used as reference.

NMR Studies——^{1}H-NMR spectra were recorded by a JEOL PS-100 spectrometer equipped with a temperature-variable apparatus. The accuracy of temperature is within ±1°. Tetramethylsilane (TMS) was used as an internal standard for $CDCl_3$ solution. The chemical shift measurements were reproducible within 0.5 cps. Proton noise-decoupled ^{13}C-NMR spectra were obtained at 25° with a JNM-PFT-100 ^{13}C Fourier transform NMR spectrometer (25.15 MHz) equipped with a JEC-6 spectrum computer. The chemical shifts were measured with external tetramethylsilane (TMS) standard and the accuracy was within ±0.06 ppm.

Results and Discussion

Solubility Studies

The solubility of phenobarbital in water increases with the chloral hydrate concentration as shown Fig. 1. The upward curvature of the plot clearly indicates the presence of higher order complexes other than 1:1. Similar solubility diagram patterns were obtained on the other BA with the exceptions of N-methyl series.

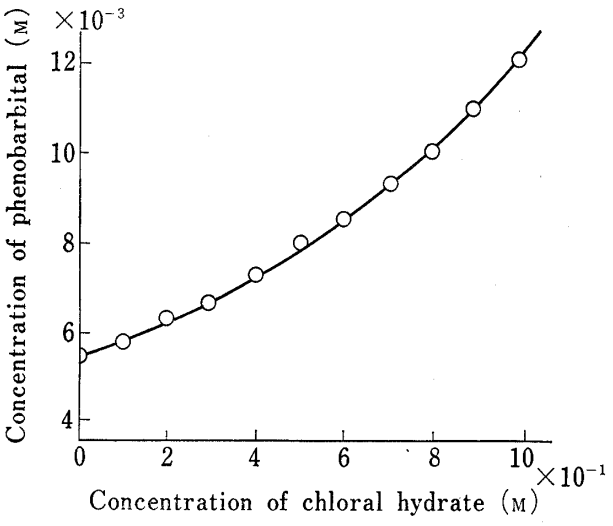


Fig. 1. Solubility Diagram of Phenobarbital in the Presence of Chloral Hydrate in Water at 25°

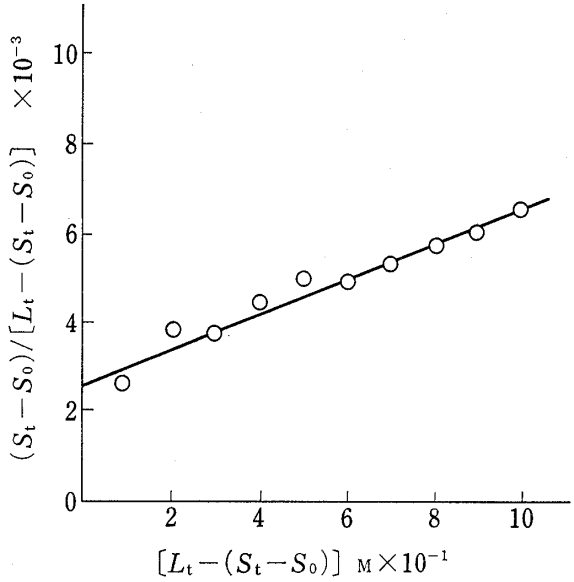


Fig. 2. Analysis of the Solubility Data for the Interaction of Phenobarbital with Chloral Hydrate in Water at 25°
See the equation 8 of Higuchi, *et al.*'s study[8]

Figure 2 shows the plots according to the equation 8 of Higuchi, *et al.* 's study[8] which assumes 1:1 and 1:2 complexes. The fairly linear plots provide the pertinence of the application of this calculation to obtain the stability constants of 1:1 and 1:2 complexes, $K_{1:1}$ and $K_{1:2}$. When the solubility increase was linear with respect to chloral hydrate concentration stability constant was calculated assuming single 1:1 complex.

7) K. Kakemi, H. Sezaki, T. Mitsunaga, and M. Nakano, *J. Pharm. Sci.*, **59**, 1597 (1970).
8) T. Higuchi and H. Kristiansen, *J. Pharm. Sci.*, **59**, 1601 (1970).

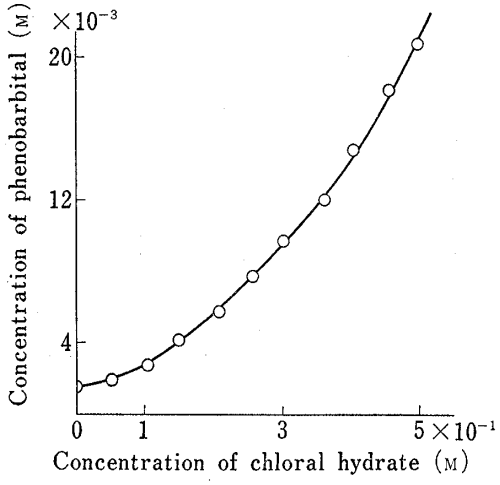


Fig. 3. Solubility Diagram of Phenobarbital in the Presence of Chloral Hydrate in Benzene at 25°

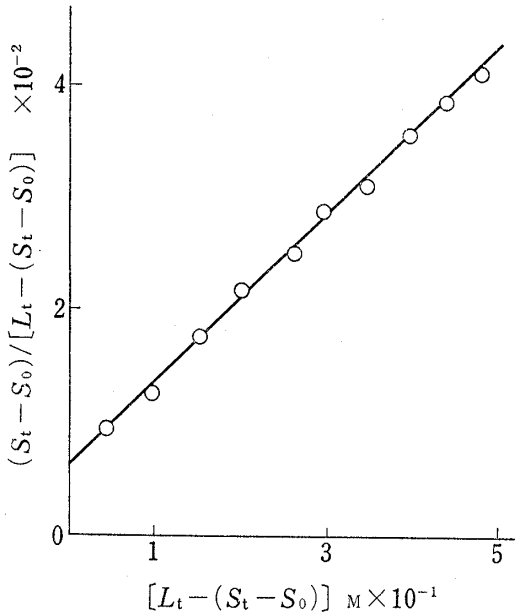


Fig. 4. Analysis of the Solubility Data for the Interaction of Phenobarbital with Chloral Hydrate in Benzene at 25°

The solubilization of BA by chloral hydrate in organic solvents were also studied. As an example, the solubilization of phenobarbital in benzene by the addition of chloral hydrate is shown in Fig. 3. Chloral hydrate is known to be dissociated in organic solvent. However, as will be later proved by NMR spectra the dissociation is markedly suppressed by the complexation with BA. Figure 4 shows the analysis of the solubility data of Fig. 3 as was carried out previously.

Stability constants obtained in water and benzene are presented in Table I. The stability constants in benzene are larger than those in water, indicating that the interaction is stronger in organic phase. It is particularly noteworthy that in water $K_{1:2}$ is larger than $K_{1:1}$. For N-methyl series the solubilities increased linearly with chloral hydrate concentration both in water and benzene and $K_{1:2}$ values were not calculable if any. This may be due to the steric hindrance of methyl group. For amobarbital and pentobarbital $K_{1:2}$ values were calculated in water but not in benzene. In benzene appreciable $K_{1:2}$ values were obtained when C-5 substituents are cyclic or short alkyl. These facts may be explained by the bending of alkyl

Table I. Stability Constants for Interaction of Barbituric Acids with Chloral Hydrate in Water and in Benzene at 25°

Compound	in water		in benzene	
	$K_{1:1}$	$K_{1:2}$	$K_{1:1}$	$K_{1:2}$
Barbital	0.46	1.38	8.78	6.97
Amobarbital	0.46	1.37	23.8	*a*
Pentobarbital	0.50	1.25	24.3	*a*
Cyclobarbital	0.57	1.18	9.08	5.94
Phenobarbital	0.48	1.46	9.68	6.40
Metharbital	0.91	*a*	6.61	*a*
Mephobarbital	0.74	*a*	6.12	*a*
Hexobarbital	1.08	*a*	6.84	*a*
Thiopental	0.51	1.06	7.84	*a*

a) Could not be determined with accuracy, may be small value if any.

C-5 substituent on pyrimidine ring which had been proved by X-ray analysis of BA crystal.[9] Inflexible cyclic substituents and short alkyl chain are not able or long enough for covering on pyrimidine ring which can be demostrated by molecular model. That the structure in organic solvent is more resemble to crystal structure comparing to that in aqueous phase is conceivable.[10] In aqueous phase the bending of alkyl chain over pyrimidine ring may not be so contiguous probably by the water structure around alkyl chain and the higher complexation may be admitted. K values for pentobarbital are larger than those for thiopental, which may be ascribed to the stronger electronegativity of oxygen comparing to that of sulfur.

Figure 5 shows the relationship between apparent $K_{1:1}$ value of phenobarbital–chloral hydrate complex and the dielectric constant of solvent, where $K_{1:1}$ value decreases with the increasing of the polarity of solvent. This indicates that the hydrogen bonding is the primary forces which bind two solutes.

Figure 6 shows the plot of $K_{1:1}$ value against pK_a of proton donors where K value decreases with pK_a. This also supports the hydrogen bonding interaction. $K_{1:1}$ values of chloral hydrate are larger than general trend, which shows that chloral hydrate is extraordinarily reactive.

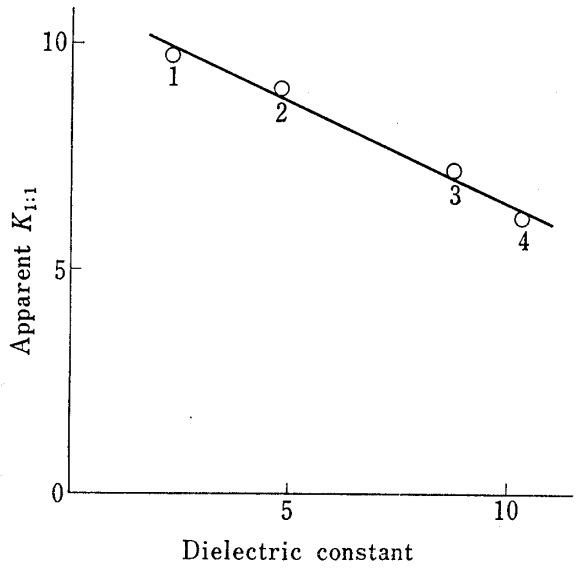


Fig. 5. Relationship between Apparent $K_{1:1}$ Value of Phenobarbital–Chloral Hydrate Complex and Dielectric Constant of Solvent at 25°

1, C_6H_6; 2, $CHCl_3$; 3, CH_2Cl_2; 4, $C_2H_4Cl_2$

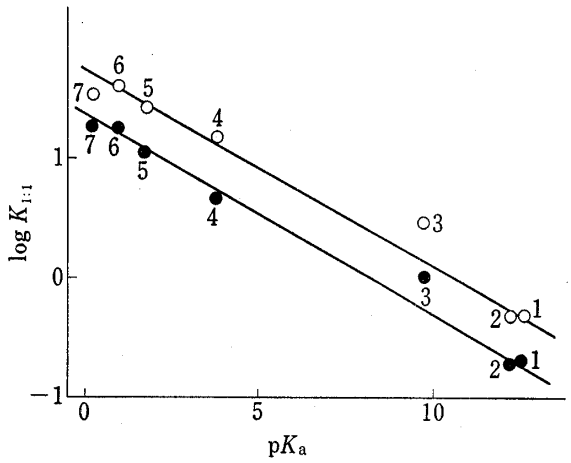


Fig. 6. Relationship between $K_{1:1}$ Value and pK_a of Proton Donors

○—○: phenobarbital—acid system, ●—●: mephobarbital — acid system

key: 1: trifluoroethanol, 2: trichloroethanol, 3: chloral hydrate, 4: monochloroacetic acid, 5: dichloroacetic acid 6: trichloroacetic acid, 7: trifluoroacetic acid

$K_{1:1}$ values were determined in benzene at 25°.

Spectroscopic Studies

Figure 7 shows the IR spectra of phenobarbital–chloral hydrate system in $CHCl_3$. The intensities of the OH stretching at 3580 and 3545 cm^{-1} decrease by the addition of phenobarbital. Accompanying to this a band appears around 3480 cm^{-1} which may be the band of hydrogen bonded OH. On the contrary, the NH stretching band of phenobarbital hardly changes in the presence of chloral hydrate, suggesting that the NH group is not involved in hydrogen bonding. The change of carbonyl bands of phenobarbital in the presence of chloral hydrate could not be detected because of the partial dissociation of chloral hydrate in organic

9) B.M. Craven, E.A. Vizzini, and M.M. Rodringues, *Acta. Cryst.*, **B25**, 1978 (1969); B.M. Craven and E.A. Vizzini, *ibid.*, **B25**, 1993 (1969).

10) H. Yokoi and T. Isobe, *Bull. Chem. Soc. Japan*, **46**, 447 (1973).

solvent. IR spectra changes were observed also on trichloroethanol–phenobarbital system where changes were smaller than those by chloral hydrate as is expected from the smaller K values.

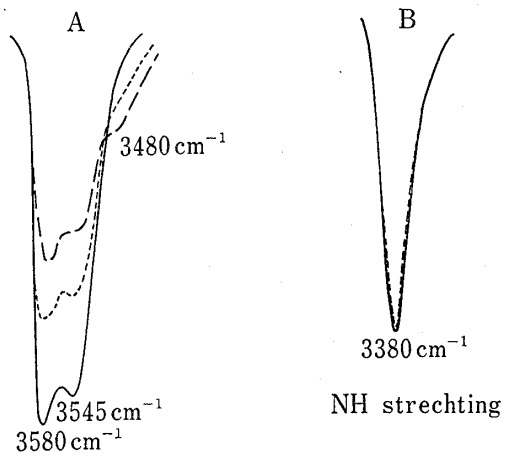


Fig. 7. IR Spectra of Phenobarbital–Chloral Hydrate System in $CHCl_3$

A: ——; chloral hydrate (0.01M) alone, ·····; chloral hydrate (0.01M)+phenobarbital (0.02M), – – –; chloral hydrate (0.01M)+phenobarbital (0.06M)
B: ——; phenobarbital (0.02M) alone, ····· phenobarbital (0.02M)+chloral hydrate (0.06M)

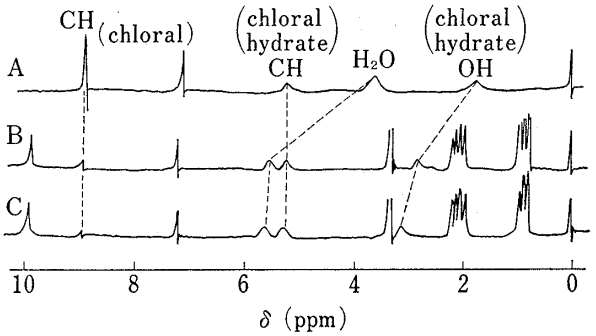


Fig. 8. Effect of Metharbital on ^{1}H-NMR Spectra of Chloral Hydrate in $CDCl_3$

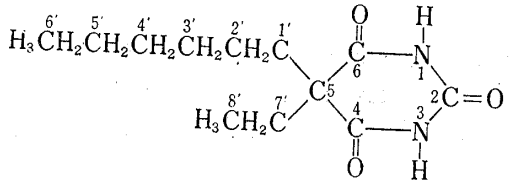
A: chloral hydrate (0.1M) alone, B: chloral hydrate (0.1M)+metharbital (0.04M), C: chloral hydrate (0.1M)+metharbital (0.06M)

Figure 8 shows the effect of metharbital on ^{1}H-NMR spectrum of chloral hydrate at −30°. The OH protons of chloral hydrate could not be detected at room temperature. However at −30° the exchange rate of the chloral hydrate protons with water becomes slow and the OH protons are clearly assigned as seen in Fig. 8. The proton signals of OH and H_2O were shifted to lower fields in the presence of metharbital. The shift of OH proton of chloral hydrate may be attributed to the hydrogen bonding with metharbital and the shift of H_2O proton may be ascribed to the rearrangement of water or the change of environment. It is noteworthy that CH proton of dissociated chloral markedly decreases upon the addition of metharbital. This indicates that the dissociation of chloral hydrate is suppressed by the BA–chloral hydrate complexation.

These interaction were further studied by ^{13}C-NMR spectra in $CDCl_3$. As is seen in Table II, chemical shifts[11] of 2- and 4- or 6-carbonyls in the presence of chloral hydrate are shifted to lower magnetic field. The shift of 2-C signal is larger than that of 4- or 6-C signal. The former shift occurs to the farthest at the lower concentration of chloral hydrate, while the latter shift proceeds as chloral hydrate increases. These results may indicate that 2-carbonyl has preferably hydrogen bonded capability and 4- or 6-carbonyl is successively hydrogen bonded. According to the molecular orbital study of Pullman, *et al.*,[12] the net electronic charge of oxygen atom in 2-carbonyl is higher than those in 4- and 6-carbonyl groups, implying that 2-carbonyl is more susceptible to the hydrogen bonding comparing to 4- or 6-carbonyl. Meanwhile, the shifts of substituent carbons were not observed in the presence of chloral hydrate.

11) A. Fratiello, M. Mardirossian, and E. Chauez, *J. Mag. Reson.*, **12**, 221 (1973); J. Okada and T. Esaki, *Chem. Pharm. Bull.* (Tokyo), **22**, 1580 (1974).
12) B. Pullman, J.L. Coubeils, and Ph. Courriere, *J. Theor. Biol.*, **35**, 375 (1972).

TABLE II. Effect of Chloral Hydrate on ^{13}C-NMR Spectra of Hexethal in $CDCl_3$



	C-2	C-4,6	C-5	C-1′	C-2′	C-3′	C-4′	C-5′	C-6′	C-7′	C-8′
Hexethal (0.40 M)	150.2	173.5	57.52	38.89	32.46	31.43	25.18	22.51	13.95	29.18	9.46
Hexethal (0.40 M)+ Chloral hydrate (0.15 M)	150.5 (−0.3)	173.6 (−0.1)	57.54 (−0.02)	38.89	32.50 (−0.04)	31.43	25.18	22.51	13.95	29.18	9.46
Hexethal (0.40 M)+ Chloral hydrate (0.30 M)	150.5	173.7 (−0.1)	57.52	38.89	32.46	31.40 (+0.03)	25.18	22.51	13.95	29.18	9.46

Values in parenthesis are differences of chemical shifts (δ (ppm)).

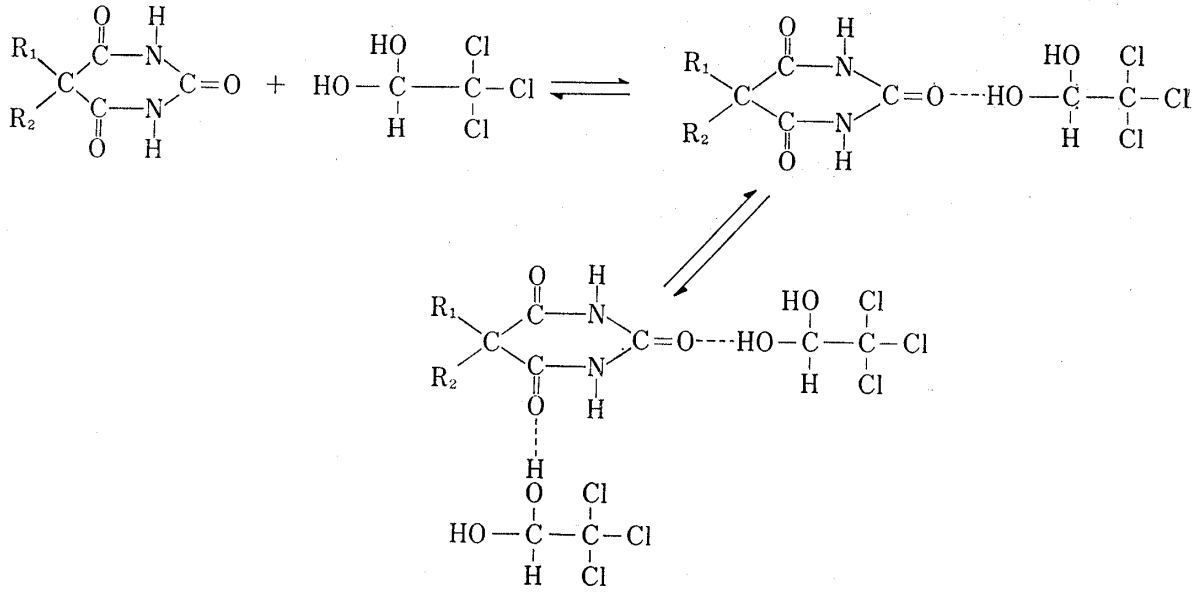


Fig. 9. Possible Structure of Complex of Barbituric Acids with Chloral Hydrate

Referring to above observations a possible structure of BA–chloral hydrate complex may be illustrated as shown in Fig. 9 where 2-carbonyl group of BA forms the hydrogen bonding with chloral hydrate precedingly and the hydrogen bonding of 4- or 6-carbonyl group takes place succeedingly. BA has three carbonyl and 1:3 or higher order complex is conceivable, but those were not detectable from the experimental results.

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