The precipitated solid mass was collected by filtration and recrystallized from acetone to give IV′ (0.15 g) as colorless prisms, mp 265°. *Anal.* Calcd. for $C_{11}H_9O_3NS$: C, 56.16; H, 3.85; N, 5.95. Found: C, 56.36; H, 4.12; N, 6.07. The filtrate gave 0.02 g of unchanged IV.

Catalytic Reduction of IV and IV′——A mixture of IV (0.5 g) and Raney Ni (1.0 g) in MeOH (50 ml) was shaken with hydrogen at room temperature. During the reduction, IV dissolved to give a clear colorless solution, and the reduction was completed within 30 min. The catalyst was filtered off and the filtrate was concentrated under reduced pressure. The resulting residue was recrystallized from MeOH to give 2-methoxycarbonylmethyl-3,4-dihydro-3-oxo-2H-benzo-1,4-thiazine (V) as colorless needles, mp 145°. Yield, 0.4 g. This compound was identified by comparing its infrared spectrum with that of an authentic sample.[5]

In a similar manner, IV′ (100 mg) was reduced to give V (75 mg).

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Complexation of Chlorpromazine with Adenosine and Its Phosphates[1]

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The molecular basis for the lowering action of chloroprmazine (CPZ) on the membrane permeability has not been cleared yet. The surface activity of CPZ itself has been thought to be ascribable to the permeability reduction for some time.[3] However, Blei[4] has demonstrated a marked decrease in the surface tension of solutions of CPZ in the presence of adenosine-5′-triphosphate (ATP), and suggested that the formation of CPZ–ATP complex of greater surface activity than CPZ alone is attributed to the reduction of membrane permeability. Hence, in the present work, the formation of complexes of CPZ with adenosine, adenosine-5′-monophosphate (AMP), and ATP has been investigated by the aid of ultraviolet absorption spectra.

Experimental

Materials——Adenosine, AMP, and ATP were of reagent grade, Ajinomoto Co., Ltd. CPZ (hydrochloride) was kindly supplied by Shionogi & Co., Ltd. They were used without further purification. Phosphate buffer (0.1M, pH 6.5) was used for the solvent unless otherwise described.

Measurement of Absorption Spectra——The absorption spectra were recorded with a Hitachi EPS-3T spectrophotometer in 0.5 cm cells at room temperature.

Determination of Equilibrium Constant——Hitachi model 139 spectrophotometer was employed for the measurements of absorbance decrease at 307 mμ of 10^{-3}M CPZ in the presence of 4×10^{-3}—4×10^{-2}M adenosine, AMP, or ATP at 25° and 37°. The values of equilibrium constant, K_C, for the complex formation were estimated by the aid of the Benesi-Hildebrand method.[5] Free energy change, ΔG, for the complexation was calculated from the value of K_C.

1) *a*) This forms Part XI of "Spectroscopic Studies on Molecular Interactions"; *b*) Part X: I. Moriguchi, S. Fushimi, and N. Kaneniwa, *Chem. Pharm. Bull.* (Tokyo), **20**, 411 (1972).
2) Location: *Hatanodai, Shinagawa-ku, Tokyo, 141, Japan.*
3) W. Scholtan, *Kolloid-Z.*, **142**, 84 (1955); F. Vilallonga, E. Fried, and J.A. Izquierdo, *Arch. Intern. Pharmacodyn.*, **130**, 260 (1961); P.M. Seeman and H.S. Bialy, *Biochem. Pharmacol.*, **12**, 1181 (1963).
4) I. Blei, *Archiv. Biochem. Biophys.*, **109**, 321 (1965).
5) H.A. Benesi and J.H. Hildebrand, *J. Am. Chem. Soc.*, **71**, 2703 (1949); I. Moriguchi and N. Kaneniwa, *Chem. Pharm. Bull.* (Tokyo), **17**, 2173 (1969).

Result and Discussion

CPZ has a strong band at 306 mμ in 0.1 M phosphate buffer at pH 6.5. For adenosine, AMP, and ATP, absorption in the vicinity of 306 mμ is weak. Fig. 1 shows spectra of CPZ, AMP, and their mixture. It is seen that, contrary to expectation, the absorption in the vicinity of 306 mμ decreases in the addition of AMP. A similar spectral decrease was also observed in the addition of adenosine or ATP instead of AMP. This is probably due to the complex formation between CPZ and adenosines. Because the absorption decrease was found to be maximum at 307 mμ with all the cases, the concentration dependency of the 307 mμ absorption decrease was measured at 25° and 37°.

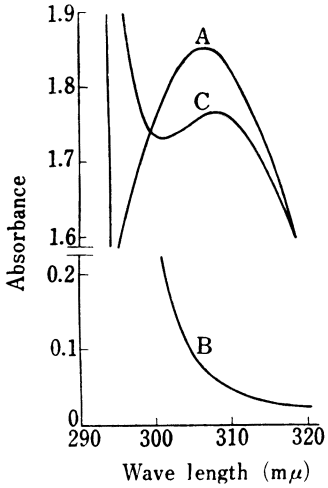

Fig. 1. Spectra of CPZ, AMP, and Their Mixture in 0.1 M Phosphate Buffer at pH 6.5
A: CPZ (10^{-3} M), B: AMP (4×10^{-2} M), C: CPZ (10^{-3} M) plus AMP (4×10^{-2} M) optical path length: 5 mm

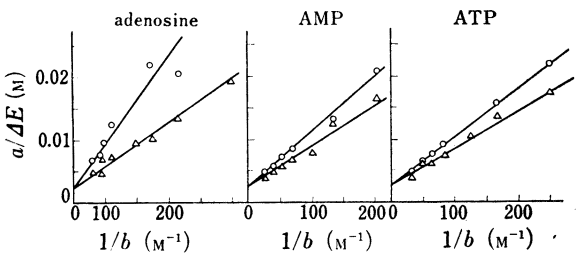

Fig. 2. Benesi-Hildebrand Plots for CPZ-Adenosine Complexation in 0.1 M Phosphate Buffer at pH 6.5
—△—: at 25° —○—: at 37°

Fig. 2 shows the Benesi-Hildebrand plots for the data, the following equation[5] being satisfied.

$$\frac{a}{\Delta E} = \frac{1}{b\cdot l\cdot \Delta\varepsilon\cdot K_C} + \frac{1}{l\cdot\Delta\varepsilon}$$

Here, a and b are the total concentrations of CPZ and adenosines, respectively, l is the length of the optical path, ΔE the 307 mμ absorbance decrease, and $\Delta\varepsilon$ is shown as

$$\Delta\varepsilon = (\varepsilon_A+\varepsilon_B) - \varepsilon_C$$

where ε_A, ε_B, and ε_C are the molar extinction coefficients at 307 mμ of CPZ, adenosines, and the complexes, respectively. This result may lead to the conclusion that 1:1 complexes are formed between CPZ and adenosines.

By the aid of the Benesi-Hildebrand relation, equilibrium constant, K_C, was estimated. Then, from the temperature dependency of K_C, apparent changes of enthalpy, ΔH, and of entropy, ΔS, were calculated. These values are listed in Table I. It can be seen that K_C increases with increasing chain length of the phosphate moiety of adenosines, although the presence of phosphate moiety is not critical for the complexation. This may suggest that ion pairing between the phosphate moiety and the tail nitrogen of CPZ is ascribable to the complexation. The higher values of ΔS for AMP and ATP as compared with adenosine may provide additional evidence for the ion pairing.

Besides the ion pairing, some specific electrostatic forces or charge-transfer forces strengthened by plane-to-plane stacking[6] seem to contribute to the complexation because of the following findings. First, adenosine bearing no phosphate moiety can form complex with

6) P. Song, W.C. Herndon, and J. Feuer, *Arch. Biochem. Biophys.*, **136**, 574 (1970).

Table I. Constants for CPZ-Adenosine Complexations in 0.1M Phosphate Buffer at pH 6.5

Adenosines	K_C (M^{-1}) 25°	K_C (M^{-1}) 37°	ΔH (kcal/mole)	ΔS (e.u.)
Adenosine	35.7	18.2	−10.3	−27.5
AMP	37.0	27.8	−4.4	− 7.5
ATP	41.0	32.3	−3.7	− 4.9

CPZ fairly well as indicated in Table I. Second, guanosine, which is a pyrimidine base similar to adenosine, was not recognized to form complex with CPZ spectrophotometrically. Furthermore, the free energy change for the complexation between adenosine and CPZ increases with increasing concentration of ethanol (Fig. 3); this may support for plane-to-plane stacking and charge-transfer,[7] and not for hydrogen bonding. Additional explanations for the charge-transfer would be that CPZ is known to be a good electron donor,[8] and that adenosine is superior in electron-accepting ability to guanosine judging from the calculated values of the lowest vacant molecular orbital energy.[9] However, the charge-transfer band was not observed at room temperature. Consequently, the detailed mechanism for the interactions remains unknown.

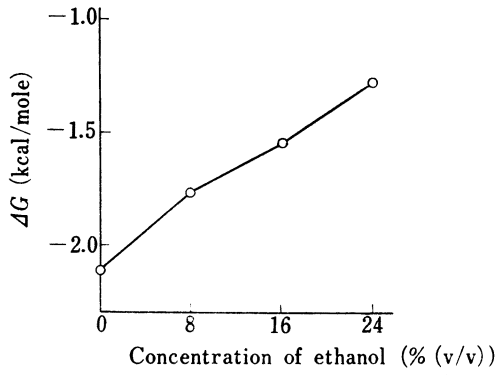

Fig. 3. Influence of Ethanol on Free Energy Change for Complexation of CPZ with Adenosine in 0.1M Phosphate Buffer at pH 6.5 and 25°

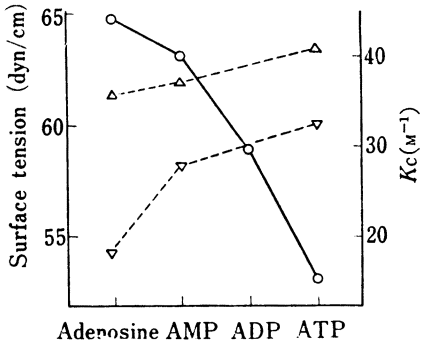

Fig. 4. K_C and Surface Tension for CPZ-Adenosine Systems

—○—: surface tension for mixed solution of CPZ (5×10^{-4}M) with adenosines (8×10^{-4}M) at pH 3.0, Ref. 4.
---△---: K_C at 25°
---▽---: K_C at 37°

Fig. 4 shows K_C and surface tension[4] for CPZ-adenosine systems. It can be seen that surface tension decreases with increasing K_C value. This may give a support for already mentioned Blei's suggestion[4] that a surface-active CPZ–ATP complex is formed which would be concerned with the action of CPZ on the membrane permeability.

7) I. Moriguchi, S. Fushimi, and N. Kaneniwa, *Chem. Pharm. Bull.* (Tokyo), **20**, 258 (1972).
8) L.E. Lyons and J.C. Mackie, *Nature*, **197**, 589 (1963).
9) T.A. Hoffmann and J. Ladik, "The Structure and Properties of Biomolecules and Biological Systems," ed. by J. Duchesne, Interscience Publishers, Inc., New York, 1964, p. 84.