CHEMICAL & PHARMACEUTICAL BULLETIN

Vol. 15 No. 11 **November 1967**

[Chem. Pharm. Bull. 15(11)1621~1625(1967)]

UDC 615.754.5-011

205. **Teisuke Okano, Kanji Aita, and Ken Ikeda**[*1]: Electronic Properties of N-Heteroaromatics. XII.[*2] On the Mode of Interaction between Theophylline and Ethylenediamine in Aqueous Solution.

(*Pharmaceutical Institute, Tohoku University School of Medicine*[*1])

With the object of obtaining information about the behavior of aminophylline in aqueous medium, the mode of interaction between theophylline (TP) and ethylenediamine (ED) in water was studied. The molar ratio of the solubilizate-solubilizer complex was confirmed to be 1:1 by means of refractometry as well as phase diagram study, and stability constant of the complex was determined by solubility method. The comparative study of TP and structurally related compounds suggested that the soluble complex is united in such a way that ionic bonding between ionized imino and amino groups of TP and ED, respectively, is stabilized by hydrogen bonding between 6-keto group of TP and amino group of ED.

(Received July 21, 1966)

Aminophylline is one of the solubilized forms of theophylline (TP) and is appreciated for its distinctively higher water-solubility than TP. The composition of aminophylline in solid state has been established to be approximately two moles of TP, one mole of ethylenediamine (ED), and two moles of water. However, no study has come out dealing with the behavior of aminophylline in aqueous solution.

In order to obtain any information about the mode of interaction between TP and ED molecules in solution, the authors compared the solubilization properties of TP with those of structurally related compounds, *viz.* theobromine, caffeine, hypoxanthine, adenine, and 8-hydroxyquinoline. The composition and stability constant of the TP-ED complex were determined by means of solubility study, refractometry, and phase diagram study. And on the basis of the results obtained, the authors presumed the way of binding of the two molecules, TP and ED.

Results and Discussion

Solubilization of Theophylline and Related Compounds

Fig. 1 shows solubilities of TP and related compounds in the presence of various concentrations of ED. Solubilities of TP, theobromine, hypoxanthine, adenine, and 8-hydroxyquinoline seem to increase with increasing concentration of ED. Caffeine is the only exception that is not solubilized by ED. However, in interpreting these data, pH change brought about by the addition of ED, which may affect the solubilities of weak acids, must be taken into account.

[*1] Kita-yobancho, Sendai (岡野定輔, 相田侃次, 池田憲).

[*2] Part XI: Yakugaku Zasshi, 87, 1243 (1967).

NII-Electronic Library Service

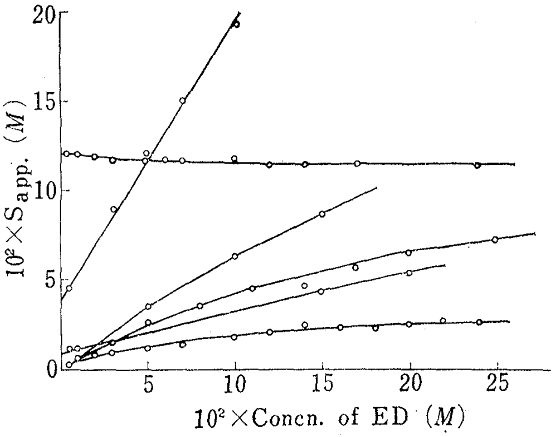

Fig. 1. Apparent Solubility of Theophylline and Related Compounds in the Presence of Ethylenediamine

Curves :
1, Theophylline
2, Caffeine
3, Hypoxanthine
4, 8-Hydroxyquinoline
5, Adenine
6, Theobromine

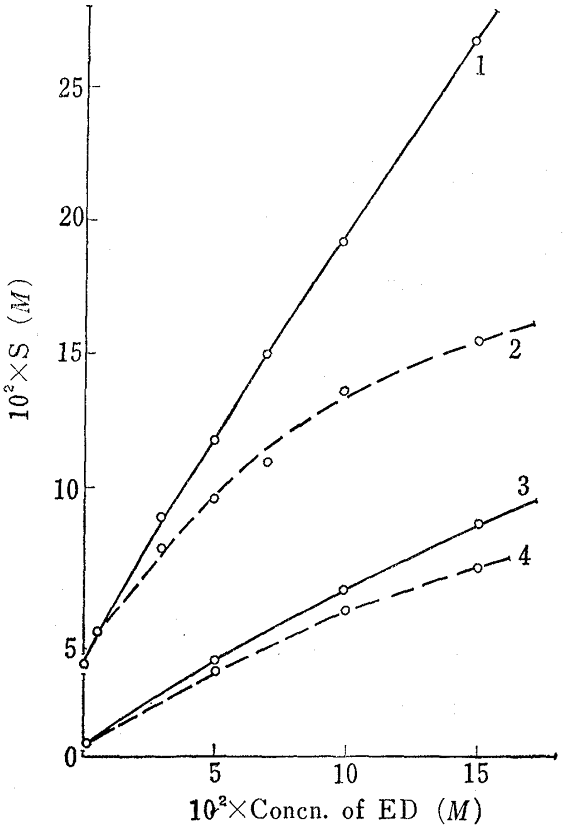

Fig. 2. Apparent and Calculated Solubilities of Theophylline and Hypoxanthine in the Presence of Ethylenediamine

Solid lines : apparent solubility.
Dotted lines : calculated solubility.
Curves : 1 and 2, theophylline.
3 and 4, hypoxanthine.

Solubility of a weak acid at a given hydrogen ion concentration, (H^+), can be evaluated by the following equation.

$$\text{Total solubility} = (S)_0\left\{1+\frac{K_1}{(H^+)}+\frac{K_1K_2}{(H^+)^2}+\cdots\cdots\right\} \qquad (1)$$

where $(S)_0$ stands for solubility of unionized species of the acid, and K_1 and K_2 stand for the first and the second dissociation constants of the acid, respectively. $(S)_0$ can be calculated from solubility of the acid and hydrogen ion concentration of sulution.

In order to evaluate the net increase of solubility of the acid caused by the addition of ED, solubility values estimated by equation (1) for given pH's must be deducted from the apparent solubility values of the acid. It was revealed from these criticisms that among the apparent solubility data of the five compounds only those for TP and hypoxanthine were significantly larger than the calculated values for given pH's. Fig. 2 shows the comparison of apparent solubility curves (solid lines) of TP and hypoxanthine (dotted lines) estimated from equation (1). The differences between the solid line and the dotted line correspond to the net increase due to the presence of ED. In calculation by equation (1), dissociation constant values of 2.51×10^{-9} for TP,[1] 1.07×10^{-10} for

---

[1] A. S. Ogston : J. Chem. Soc., **1935**, 1376.

theobromine,[2] 6.03×10^{-5} and 1.59×10^{-10} for adenine,[3] 1.05×10^{-2} and 1.15×10^{-9} for hypoxanthine,[4] and 1.20×10^{-5} and 1.95×10^{-10} for 8-hydroxyquinoline[5] were used.

Refractometry of Mixed Solutions of TP and ED

Fig. 3 shows relationship between square of refractive index and molar ratio of TP and ED in aqueous solution. The result could be taken as indication of formation of complex of 1:1 molar ratio, which is in accord with the fact confirmed with a ternary phase diagram as described below.

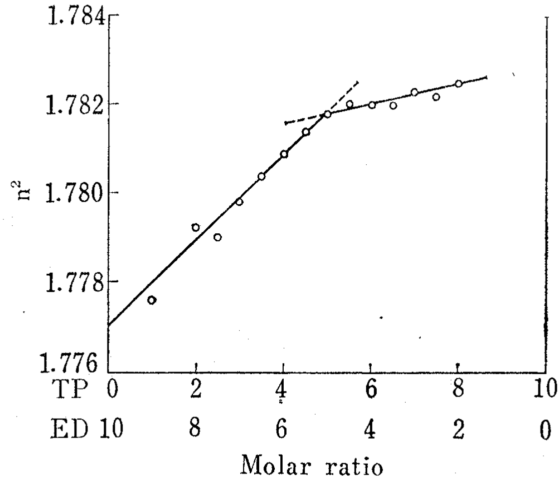

Fig. 3. Relationship between Square of Refractive Index and Molar Ratio of Theophylline and Ethylenediamine

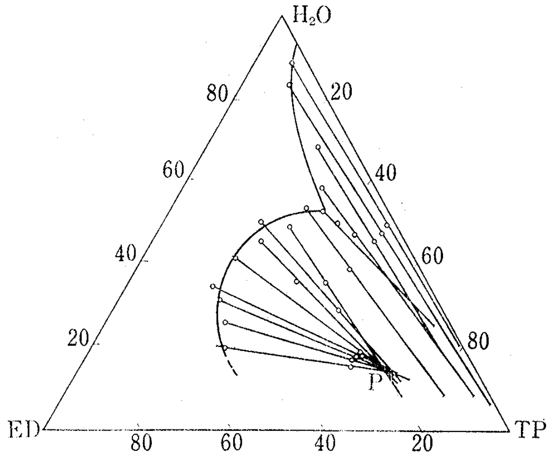

Fig. 4. Ternary Phase Diagram for Theophylline-Ethylenediamine-Water System

Ternary Phase Diagram of TP-ED-H_2O

Fig. 4 is a ternary phase diagram of TP, ED, and water shown in percentage by weight. Mixtures of low water content and/or high ED content were too viscous to obtain reliable results. The diagram has distinct phase boundary composed of two curves, but at high content of ED the tie lines converge into the point P, whose composition is one mole of TP, one mole of ED, and two moles of water.

Stability Constant of the Complex

Assuming that ionic bonding between TP and ED does participate in the formation of the 1:1 complex in aqueous solution, stability constant could be calculated as follows. Since ED is diacidic, it is necessary to compute two stability constants, k_1 and k_2. These may be expressed by equations (2) and (3), where (T^-), (E^+) and (E^{2+}) represent concentration of TP ion, monovalent ED ion, and divalent ED ion, respectively.

$$k_1=\frac{(TE)}{(E^+)(T^-)} \tag{2}$$

$$k_2=\frac{(TE^+)}{(E^{2+})(T^-)} \tag{3}$$

(TE) and (TE^+) stand for concentration of complexes of TP with monovalent ED and divalent ED ions, respectively. Each term of the right side of equations (2) and (3) can be known from the following stoichiometric equations (4)~(9).

---

[2] L.F. Cavalieri, J.J. Fox, A. Stone, N. Chang: J. Am. Chem. Soc., **76**, 1119 (1954).
[3] H.F.W. Taylor: J. Chem. Soc., **1948**, 765.
[4] A. Albert, D.J. Brown: *Ibid.*, **1954**, 2060.
[5] J.P. Philips: L.L. Meritt: J. Am. Chem. Soc., **70**, 410 (1948).

$$\text{Total concn. of TP} = (\text{TH}) + (\text{T}^-) + (\text{TE}) + (\text{TE}^+) \qquad (4)$$

$$\text{Total concn. of ED} = (\text{E}) + (\text{E}^+) + (\text{E}^{2+}) + (\text{TE}) + (\text{TE}^+) \qquad (5)$$

$$k_{\text{E1}} = \frac{(\text{E}^+)(\text{H}^+)}{(\text{E}^{2+})} \qquad (6)$$

$$k_{\text{E2}} = \frac{(\text{E})(\text{H}^+)}{(\text{E}^+)} \qquad (7)$$

$$k_{\text{T}} = \frac{(\text{T}^-)(\text{H}^+)}{(\text{TH})} \qquad (8)$$

$$k_{\text{C}} = \frac{(\text{TE})(\text{H}^+)}{(\text{TE}^+)} \qquad (9)$$

In these equations (TH) and (E) represent concentration of unionized species of TP and ED, respectively. k_{E1} and k_{E2} are dissociation constants of ED, which were found to be 1.00×10^{-7} and 8.13×10^{-11} by Bertsch, *et al.*⁶⁾ k_{T} is dissociation constant of TP.¹⁾ Hydrogen ion concentration, (H^+), and total concentration of TP and ED can be determined directly. (TH) was found to be $3.95 \times 10^{-2} M$ at 25°, which was constant despite of the presence of excess TP. k_{C}, dissociation constant of TE^+, could reasonably be assumed to be equal to k_{E1}. Table I shows the calculated values for each term and stability constant. The relatively uniform values of stability constants shown in the table could be taken as support for the above-mentioned discussions.

Table I. Calculation of Stability Constants of TP–ED Complexes (25°)

Concn of ED ($10^{-2}M$)	$S_{\text{app.}}$ of TP ($10^{-2}M$)	pH found	Concn. of complex ($10^{-2}M$)	(TE) ($10^{-2}M$)	(TE^+) ($10^{-2}M$)	(T^-) ($10^{-2}M$)	(E^+) ($10^{-2}M$)	(E^{2+}) ($10^{-4}M$)	k_1	k_2
0	3.95	6.08	—	—	—	—	—	—	—	—
0.5	4.62	7.93	—	—	—	—	—	—	—	—
3.0	9.16	8.61	1.19	1.16	0.029	4.03	1.71	4.21	16.9	21.0
5.0	12.0	8.77	2.24	2.22	0.038	5.82	2.11	3.59	18.1	18.0
7.0	15.2	8.87	3.94	3.90	0.053	7.32	2.84	3.83	18.8	19.5
10.0	19.5	9.00	5.66	5.61	0.056	9.90	3.97	3.97	14.3	14.3
15.0	26.9	9.08	11.06	11.0	0.092	11.9	3.56	2.97	26.0	25.8

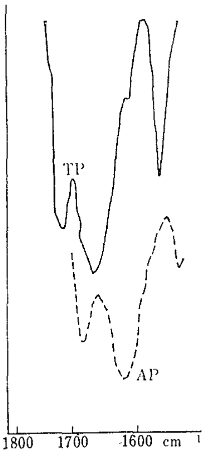

Fig. 5. Comparison of IR Spectra of Aminophylline (AP) and Theophylline (TP) in Nujol

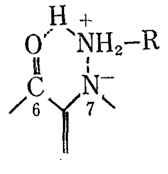

Chart 1.

Structural Considerations

It is obvious from the discussion mentioned above that ionic bonding takes part in the complex formation between TP and ED. However, it may be sure that energy of ionic bonding alone is insufficient to maintain two counterions in a stable complex in polar solvent such as water. The results shown in Fig. 1 are noticeable in this regard: there is a distinct difference between chemical structures of the compounds which are solubilized by ED and those of the compounds which are not solubilized by ED. While the formers have carbonyl group at position-6 and imino group at position-7 concurrently, the latters are devoid of one or both of such groups. The necessity of both groups for solubilization does inevitably suggest the cooperative functions of these groups. And it may be quite within the range of possibility that the solute and the

---

6) C.R. Bertsch, W.C. Ferinelius, B.P. Block: J. Phys. Chem., **62**, 444 (1958).

solubilizer may unite in such a way that ionic bonding between ionized imino and amino groups of TP and ED, respectively, is stabilized by hydrogen bonding between 6-keto group of TP and amino group of ED, as expressed in Chart 1. The difference between frequencies of C=O stretching vibration of TP (1680 cm^{-1}) and aminophylline (1625 cm^{-1}) in Nujol could also be taken as support for participation of 6-keto group in the complex formation (Fig. 5).

Materials and Methods

Materials——TP and caffeine of guaranteed grade were recrystallized from water. TP, m.p. 270~272°; caffeine, m.p. 237~238°. Theobromine, adenine, hypoxanthine, and 8-hydroxyquinoline of guaranteed grade were used without further purification. ED of guaranteed grade was boiled before use and cooled in a flask stoppered with a soda lime tube.

Measurement of Solubility——15 ml. of ED solution of appropriate concentration and excess solubilizate were taken in a 20 ml. ampule and sealed. Ampules were mounted on a water thermostat adjusted to 25 ± 0.1° and incubated for 36 hr., with occasional shaking. Test solutions were taken by a pippete equipped with a cotton filter and diluted with appropriate amount of acetate buffer solution (HCl-acetate buffer in the case of 8-hydroxyquinoline), and analyzed for solute by UV spectrophotometry. Wavelengths used were 272, 272, 272, 245, 249, and 249 mμ for TP, caffeine, theobromine, adenine, hypoxanthine, and 8-hydroxyquinoline, respectively. pH of buffer solutions was adjusted to 5.0 except in the case of 8-hydroxyquinoline (pH 2.33), which was insoluble at pH 5.0.

Refractometry——Abbe's refractometer supplied by Shimadzu Seisakusho was used. Total concentration of serial mixtures of TP and ED was kept always at $0.1M$. Solutions were stored for 24 hr. before use to obtain equilibrium.

Ternary Phase Diagram——Weighed amounts of TP, ED, and water were taken in a 20 ml. ampule and sealed. Before mounting the ampules on a thermostat, they were warmed in a water bath of about 60° for several hr. to dissolve almost of the solid. Without this pre-warming, undissolved phase of TP was coated with newly formed masses and was prevented from further dissolution. TP was determined spectrophotometrically, and ED by titration with standardized HCl solution. For analysis of the solid phase, sample was centrifuged to swish liquid phase off. Amount of water was estimated by subtracting the sum of the found quantities of TP and ED from total amount of sample.

NII-Electronic Library Service