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19. **Harutada Negoro, Tomonori Miki, and Shogo Ueda:** Interaction between Pyrazinamide and Sodium *p*-Aminosalicylate or Sodium Hydroxybenzoates in Aqueous Solution.[1]

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Pyrazinamide, an antituberculosis agent, is slightly soluble in water, the solubility being only about 16 mg./g. at ordinary temperature (25°±0.2°)[2] and it is therefore difficult to prepare its highly concentrated aqueous solution. The present study deals with a spectrophotometric determination of equilibrium constants of 1:1 complexing reaction between pyrazinamide and solubilizing agents, such as sodium *p*-aminosalicylate (PAS-Na) and sodium hydroxybenzoates (HB-Na), in aqueous solution. It has been found that the solubility of pyrazinamide in water is markedly increased by the presence of PAS-Na or HB-Na, producing a strongly colored solution in the case of the former. Aoki[3] recently confirmed through thermal analysis, the complex formation between pyrazinamide and PAS-Na.

The reaction of pyrazinamide and PAS-Na to produce the colored solution, as mentioned above, may be represented as the formation of a pyrazinamide-PAS-Na complex and equilibrium constant, K, for this reaction may be expressed as

$$K=[\mathrm{Py}]\,[\mathrm{P}]\,/\,[\mathrm{PyP}] \qquad (1)$$

where [Py], [P], and [PyP] are the concentration of free pyrazinamide, free PAS-Na, and pyrazinamide-PAS-Na complex, respectively, and observed absorbance, A, of the colored solution (1-cm. cell) can be denoted as follows:

$$A=\varepsilon\,[\mathrm{PyP}] \qquad (2)$$

where ε is the molar extinction coefficient of the complex.

In the present experimental regions, it may be considered that $P \fallingdotseq [P]$ and $Py=[Py]+[PyP]$, then Eq. (3) may be derived from Eq. (1) and (2),

$$K/\varepsilon + P/\varepsilon = PPy\,/\,A \qquad (3)$$

where P and Py are the concentration of total PAS-Na and pyrazinamide added, respectively. Therefore, linear relationship may be recongnized between P and PPy/A and the equilibrium constant can be obtained from the intercept of this line.

The colorless complexing reaction between pyrazinamide and HB-Na, in the presence of PAS-Na which forms a colored complex, would be represented as follows:

$$\begin{array}{c}\text{Pyrazinamide} + \text{HB-Na} + \text{PAS-Na} \rightleftarrows \\ \text{Pyrazinamide–HB-Na} + \text{Pyrazinamide–PAS-Na} \qquad (4) \\ \text{(colorless complex)} \qquad \text{(colored complex)}\end{array}$$

The equilibrium constant, K′, for the above colorless complex formation would be

$$K'=[\mathrm{S}]\,[\mathrm{Py}]\,/\,[\mathrm{PyS}] \qquad (5)$$

where [S] and [PyS] are the concentration of the free HB-Na and the complex, respectively.

In addition to Eqs. (1), (2), and (5), considering that $P \fallingdotseq [P]$, $S \fallingdotseq [S]$, and $Py=[Py]+[PyP]+[PyS]$ in the present experimental regions, Eq. (6) can be derived as follows:

$$K'=KSA\,/\,\{\varepsilon PPy-KA-PA\} \qquad (6)$$

If the experimental values of S, P, PyA, and K satisfy the Eq. (6), the complex formation may be concluded and the equilibrium constant K′ can be calculated from this equation.

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[1] A part of this paper was read at the 78th Annual Meeting of the Pharmaceutical Society of Japan in Nagoya, April, 1958.

[2] Paper presented at the 77th Annual Meeting of the Pharmaceutical Society of Japan in Tokyo, April, 1957.

[3] M. Aoki: Private communication.

Experimental

Material—Pyrazinamide and PAS-Na were all commercial products (pure crystals of Sankyo Co.). Sodium *o*-, *m*-, and *p*-hydroxybenzoate were also commercial products (G. R., Tokyo Kasei Co., Ltd.).

Preparation of Samples—Materials were weighed accurately into a 50-cc. volumetric flask and dissolved in water to make solutions of desired concentrations.

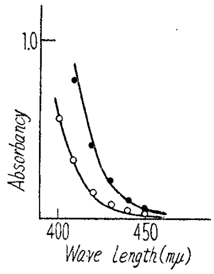

Fig. 1. Absorption Spectra of Pyrazinamide-PAS-Na in Aqueous Solution

—●— Py: 0.0812 mol./L.
P : 1.14 mol./L.
—○— Py: 0.0406 mol./L.
P : 0.571 mol./L.

Absorption Spectra—Absorption spectra in the visible region of the following solutions are shown in Fig. 1. Curves in Fig. 1 are the spectra of typical solutions of pyrazinamide and PAS-Na in water. Values in Tables I and II are the absorbancies of solutions of pyrazinamide and PAS-Na under various conditions. Values in Table IV are the absorbancies of aqueous solutions of pyrazinamide, PAS-Na, and sodium hydroxybenzoates. The absorbancy was determined at 400 and 410 mμ using Beckman Model DU spectrophotometer at 25°.

TABLE I. Absorbance of Pyrazinamide-PAS-Na in Aqueous Solution
Py: P=0.0812: 0.512 mol./L. at 25° varying pH

pH λ (mμ)	7.8	10.0	10.7	12.4
400	0.919	0.925	0.927	0.923
410	0.506	0.520	0.505	0.514

TABLE II. Absorbance of Pyrazinamide-PAS-Na in Aqueous Solution
Pyrazinamide (Py): 0.0406 mole/L. at 25°

P(mole/L.) λ (mμ)	0.286	0.572	1.14
400	0.282	0.467	0.720
410	0.157	0.263	0.396

Py: 0.0812 mol./L.

P(mole/L.) λ (mμ)	0.286	0.572	1.14
400	0.552	0.912	0.435
410	0.306	0.506	0.786

TABLE III. Equilibrium constant (K) and Extinction Coefficient (ε) of Pyrazinamide-PAS-Na Complex in Aqueous Solution

λ (mμ)	Py(mole/L.)	K	ε	
400	0.0406	1.26	37.3	38.0
	0.0812	1.34	38.5	
410	0.0406	1.19	19.8	20.2
	0.0812	1.27	20.5	
average		1.27		

TABLE IV. Absorbance at 400 mμ of Pyrazinamide-PAS-Na and Na Hydroxybenzoates in Aqueous Solution at 25°

Py (mole/L.)	P (mole/L.)	S (mole/L.)	A_{400}		
			ortho-	*meta-*	*para-*
0.0812	0.571	0.625	0.714	0.750	0.750
0.0812	1.14	0.625	1.187	1.259	1.284
0.0812	0.571	1.25	0.585	0.670	0.720
0.0812	1.14	1.25	1.007	1.125	1.161

Solubility Determination—The saturated solution of pyrazinamide was prepared by rotating the sealed ampules containing the solvent and an excess powder of pyrazinamide for 8 hr. in a water bath maintained at 25° ± 0.1°. The solution of solubilization agents used as a solvent was prepared by measuring into a dry volumetric flask the desired agent, then diluting to the mark with water at 25°. After the solution was saturated, excess solute was removed by filtration. An aliquot for analysis was placed in a weighed flask, diluted with water, and the concentration determined spectrophotometrically (pyrazinamide: $\lambda_{\max}^{H_2O}$ 269 mμ, $E_{1cm}^{1\%}=655$).

Results and Discussion

It may be considered that the linear relationship between PPy/A and P in Fig. 2 supports the assumption that pyrazinamide reacts with PAS-Na to form 1:1 molecular complex as supposed in Eqs. (1), (2), and (3). In the present experimental region, equilibrium was independent of the pH of solution as shown in Table I, and the equilibrium constant K, shown in Table III was calculated from Eq. (3).

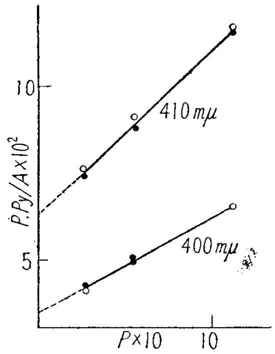

Fig. 2. Typical Plot used in the Graphical Determination of the Equilibrium Constant and Extinction Coefficient

● Py: 0.0406 mole/L.
○ Py: 0.0812 mole/L.

As shown in Table V, the equilibrium constant, K′ was independent of the concentration of Py, P, or S, and it may be considered also that pyrazinamide reacts with sodium hydroxybenzoate to form 1:1 complex as supposed in Eqs. (4), (5), and (6).

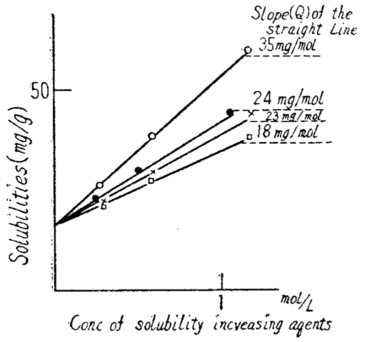

Fig. 3. Relationship between Solubility of Pyrazinamide and Concentration of Solubilizing Agents

—○— Na *o*-hydroxybenzoate
—●— PAS-Na
—×— Na *m*-hydroxybenzoate
—□— Na *p*-hydroxybenzoate

It is shown in Fig. 3 that the increased solubility of pyrazinamide is directly proportional to the concentration of PAS-Na or sodium hydroxybenzoates. The slope of this solubility curve shown in Fig. 3 is the quantity of increased solubility of pyrazinamide per mole of solubilizing agent added. The relationship between slope Q of these solubility curve and equilibrium constant K′ of sodium hydroxybenzoates may be expressed by the following simple form:

$$K' = cQ$$

This may suggest some mutual relationship between increased solubility and complex formation. Higuchi[4] explained the increased solubility of caffeine, by sodium hydroxybenzoates as the solubilizing agent, as a result of complex formation. From the values of increased solubility shown in Table VI, the equilibrium constant K″ can also be calculated from Eq. (7) according to Higuchi's method,

$$K'' = (S - Py - Pyo)Pyo/(Py - Pyo) \qquad (7)$$

where Pyo is the solubility of pyrazinamide in water, Py, the solubility of pyrazinamide in a solution of solubilizing agent, and S, the total concentration of sodium hydroxybenzoates or PAS-Na added. However, it may be easily seen that the equilibrium constant K or K′ obtained from spectrophotometric data differs from K″. This difference might be the difference of assumption in the two calculations. While the con-

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4) T. Higuchi, *et al.*: J. Am. Pharm. Assoc., Sci. Ed., **42**, 132, 139(1953).

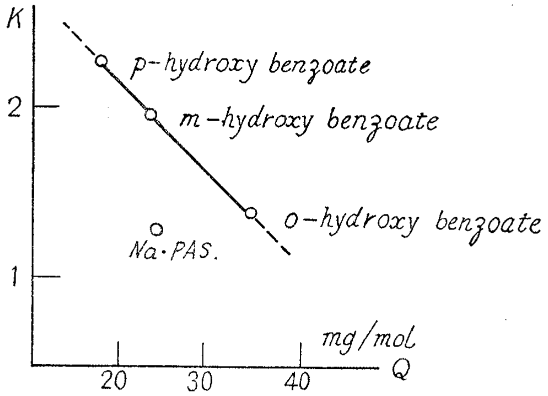

Fig. 4. Relationship between Equilibrium Constant (K) and slope (Q) of the Straight Line shown in Fig. 3.

TABLE V. Calculation of Equilibrium Constant (K′) of Pyrazinamide-Na Hydroxybenzoate Complex in Aqueous Solutions

$K' = 1.27\,A_{400}\,S/(38\,PPy - 1.27A_{400} - PA_{400})$

Py (mole/L.)	P (mole/L.)	S (mole/L.)	K′		
			ortho-	*meta-*	*para-*
0.0812	0.571	0.625	1.28	1.59	1.58
0.0812	1.14	0.625	1.44	2.08	2.43
0.0812	0.571	1.25	1.37	2.00	2.64
0.0812	1.14	1.25	1.47	2.23	2.58
average			1.39	1.98	2.31

TABLE VI. Solubility of Pyrazinamide in Various Solutions of Solubilizing Agent at 25°

Concn. of solubilizing agent (mole/L.)	Solubility of pyrazinamide (mg./g.)			
	PAS-Na	Na-*o*-HB	Na-*m*-HB	Na-*p*-HB
0.313	23a)	27	23	21
0.625	30b)	39	30	29
1.25	44c)	60	45	39

a) Concn. of PAS-Na: 0.286 mole/L.
b) Concn. of PAS-Na: 0.572 mole/L.
c) Concn. of PAS-Na: 1.14 mole/L.

TABLE VII. Equilibrium Constant (K″) of Pyrazinamide-Na Hydroxybenzoate Complex Calculated from Solubility Data

	ortho		*meta*		*para*	
S (mole/L.)	Py (mole/L.)	K″	Py (mole/L.)	K″	Py (mole/L.)	K″
0	0.13	—	0.13	—	0.13	—
0.313	0.219	0.328	0.187	0.582	0.173	0.815
0.625	0.317	0.305	0.244	0.584	0.239	0.615
1.25	0.488	0.324	0.366	0.565	0.320	0.715
K″ average		0.319		0.577		0.715

P (mole/L.)	PAS-Na	
	Py (mole/L.)	K″
0	0.13	—
0.286	0.187	0.521
0.572	0.244	0.521
0.14	0.358	0.520
K″ average		0.521

centration of free prazinamide seems to be independent of the concentration of solubilization agent, S, and equal to Pyo in the calculation of K″, the concentration of prazinamide, free form, and [Py] in its saturated solution will increase in the present experimental region, according to the amount of solubilization agent added, S, as shown in Eq. (8) which is derived from Eq. (1) or (5).

$$Py=\{-(K+S-Py)+\sqrt{(K+S-Py)^2+4KPy}\}/2 \qquad (8)$$

From these results and considerations it may be concluded that the effect of PAS-Na or sodium hydroxybenzoates in increasing the solubility of pyrazinamide in water is attributed to following two factors: One is the complex formation between pyrazinamide and PAS-Na or sodium hydroxybenzoate and another is the solubility increase of pyrazinamide (free form) in a solution of PAS-Na or sodium hydroxybenzoate. It is still not certain from the present studies which is the actual cause.

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Summary

Alkali salts of some aromatic hydroxycarboxylic acids are effective in increasing the solubility of pyrazinamide in water and the use of sodium *p*-aminosalicylate (PAS-Na) results in the formation of a yellow solution of pyrazinamide-PAS-Na complex. It has been found from examination of absorption spectrum in the visible range that the coloration is due to the formation of a 1:1 molecular compound of pyrazinamide and PAS-Na in aqueous solution. It was also found that sodium *o*-, *m*-, and *p*-hydroxybenzoates also form 1:1 molecular compound with pyrazinamide.

There is a linear relationship between increased solubility of pyrazinamide per mole of aromatic hydroxycarboxylic acid added and equilibrium constant of the molecular compound thereby formed. The equilibrium constants obtained in the present series of work were larger than the values calculated from the solubility increase and some considerations were made on the reason for this difference.

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