

130. Harutada Negoro, Shōgo Ueda, and Toshihiko Hoshi : Interaction between Pyrazine- or Pyridine-monocarboxamide and Sodium Hydroxy-carboxylate in Aqueous Solution. III.<sup>1)</sup>

(Shinagawa Plant, Sankyo Co., Ltd.\*<sup>1)</sup>)

In the preceding paper,<sup>1)</sup> it was reported that from the measurement of the heats of solution of pyrazine- or pyridine-monocarboxamide (amides) in sodium 4-amino-salicylate (Na-PAS) or sodium salicylate (Na-SAL) aqueous solution, the dissociation constants (K) of complex between them and the heats of formation ( $\Delta H_c$ ) could be estimated. In this paper, dissociation constants of the complex between above same amides and Na-PAS in aqueous solution (K) were obtained at various temperatures (T) by the absorption spectra measurement, then  $\Delta H_c$  was evaluated from the correlation between  $\log K$  and  $1/T$ . Thus obtained  $\Delta H_c$  value by spectral method exceedingly differs from that of thermal method<sup>1)</sup> considering the experimental error, so the discussion was made on this reason.

Results and Discussion

Fig. 1~5 show the relation between AP/E and P, where A is molar concentration of amide added, P is molar concentration of Na-PAS added, and E is observed absorbance of amide-PAS colored solution corrected by that of the each individual solution

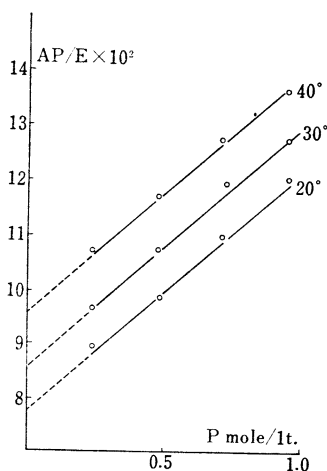


Fig. 1. Relation between AP/E and P Picolinamide-NaPAS system at 355 m $\mu$  and pH 7.0

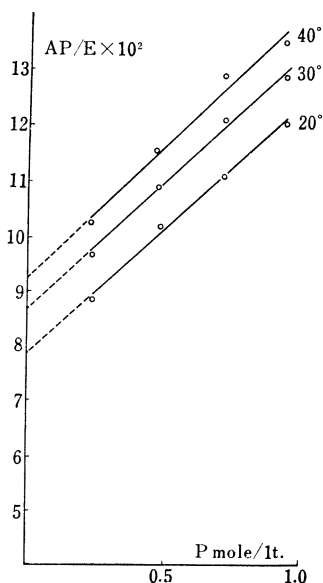


Fig. 2. Relation between AP/E and P Nicotinamide-NaPAS system at 357 m $\mu$  and pH 7.0

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1) Part II. H. Negoro *et al.*: This Bulletin, 9, 164 (1961).

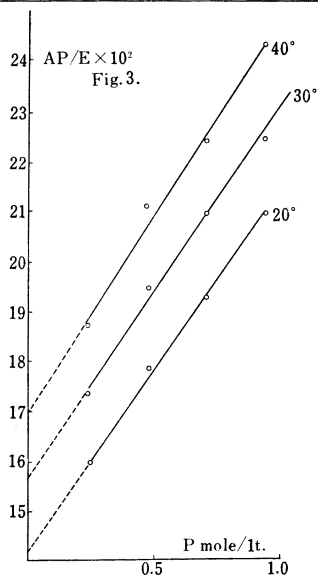


Fig. 3. Relation between AP/E and P Isonicotinamide-NaPAS system at 385  $m\mu$  and pH 7.0

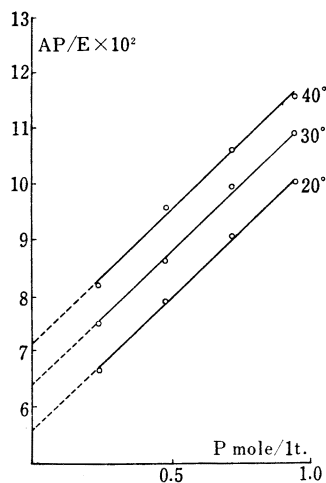


Fig. 4. Relation between AP/E and P Pyrazinamide-NaPAS system at 410  $m\mu$  and pH 7.0

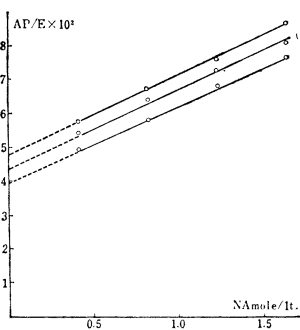


Fig. 5. Relation between AP/E and A Na-PAS-Nicotinamide system at 357  $m\mu$  and pH 7.0

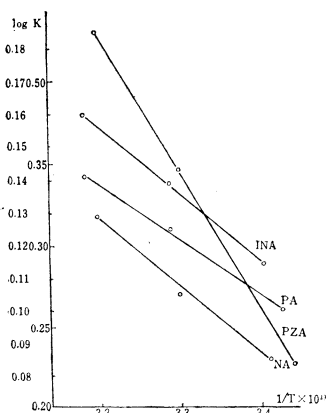


Fig. 6. Relation between  $\log K$  for Pyrazinamide and  $1/T$

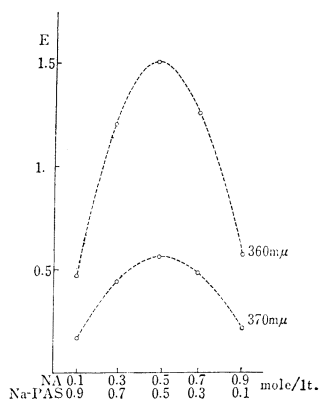


Fig. 7. Absorption Spectra of NA-NaPAS aqueous solution (continuous variation method) at pH 7.0 and 25°

of the same concentration. The relations between AP/E and P are linear in all the cases, so 1:1 complex formation are ascertained, giving K from the slope and the intercept of these lines.

In the case of Nicotinamine (NA), NA can be dissolved in water at a great extent (18% w/w at 0°, 52% w/w at 30°),<sup>2)</sup> so the measurement of the absorption spectra was done inversely of various amide concentrations such as 0.41, 0.82, 1.23, 1.64M and Na-PAS concentration was kept constant at 0.0474M. Table I shows K value with 95% confidence limits; each value is an average of repeated three experiments. In the case of NA-NaPAS system, K values obtained by the different two experiments show good coincidence, so it may be judged that the spectrophotometric measurements are suitable for checking the complex formation between amides and Na-PAS. As for the mole ratio of these complex, result of continuous variation method with NA-NaPAS system also shows 1:1 mole ratio in Fig. 7.

The relations between log K and 1/T for various amide-NaPAS complex are shown in Fig. 6, where the linear relationship are recognized in all the cases. The heat of complex formation,  $\Delta H_c$  can be calculated from the slope of these line (a) in Fig. 6 as follows:  $\Delta H_c = 2.303 R a = 4.576a$  (cal./mole).  $\Delta H_c$  thus obtained are shown in Table II, existing between 1~2 K cal./mole.

TABLE I. Dissociation Constants of Amide-NA-PAS Complex and their 95% Confidence Limits

Amide	Temp. (°C)			
	20	30		40
Picolinamide (PA)	1.86 ± 0.07	2.04 ± 0.06	1.23 ± 0.16 <sup>a)</sup>	2.20 ± 0.07
Nicotinamide (NA)	1.70 ± 0.05	1.87 ± 0.03	0.935 ± 0.32 <sup>a)</sup>	2.08 ± 0.11
	1.76 ± 0.1 <sup>b)</sup>	2.00 ± 0.09 <sup>b)</sup>		2.17 ± 0.10 <sup>b)</sup>
Isonicotinamide (INA)	1.94 ± 0.07	2.18 ± 0.07	0.69 ± 0.09 <sup>a)</sup>	2.40 ± 0.08
Pyrazinamide (PyA)	1.21 ± 0.04	1.39 ± 0.02	0.63 ± 0.23 <sup>a)</sup>	1.53 ± 0.01

a) By thermal method: This Bulletin, 9, 164 (1961).

b) From Fig. 5's data.

TABLE II. Heats of Complex Formation between Amide and Na-PAS.  $\Delta H_c$  (Kcal./mole.)

Amide	Spectral method	Thermal <sup>a)</sup> method	Amide	Spectral method	Thermal <sup>a)</sup> method
Picolinamide	1.37	3.53	Isonicotinamide	1.95	3.16
Nicotinamide	1.91 (1.97 <sup>b)</sup> )	3.65	Pyrazinamide	1.74	3.55

a) This Bulletin, 9, 164 (1961).

b) From Fig. 5's data.

Considering that both amides and Na-PAS have an aromatic ring structure, the complex formation attends changes in absorption spectra, and  $\Delta H_c$  are relatively small, it may be concluded the type of the interaction forces between amide-NaPAS complex cited above is an aromatic electron donor acceptor type.<sup>3)</sup> Comparing the  $\Delta H_c$  or K value of complex formation reported in the preceding paper<sup>1)</sup> with those shown in Table I and II, marked difference can be found, considering the experimental error. As for K value at 30°, spectral method gives a greater value than that obtained by thermal method. As for  $\Delta H_c$  value, on the contrary, thermal method gives a greater value than the spectral method.

Assuming that an another source of the liberation of heat except the complex formation which cause the absorption spectral change exists, following consideration may be made;

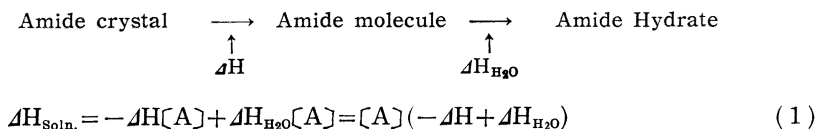
2) H. Negoro: Yakugaku Zasshi, 80, 674 (1960).

3) L. J. Andrew: Chem. Revs., 54, 713 (1954).

1. Another one or more species of complex formation with no spectral change.

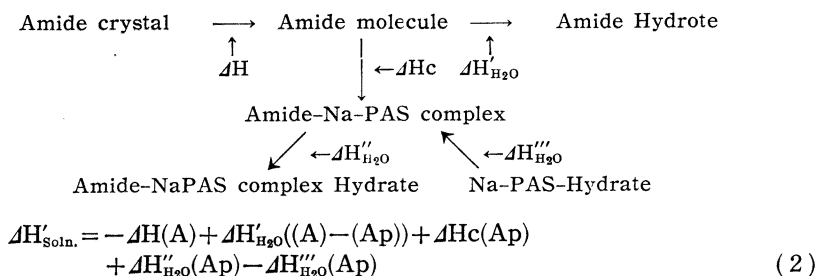
2. The liberation of heat, except the heat liberation by any complex formation between amide and Na-PAS, may be expressed as follows, considering the dissolution of amide in water or in Na-PAS aqueous solution :

(1) in water :



where  $\Delta H_{\text{soln.}}$  is heat of solution of amide in water,  $\Delta H$  is heat of vaporization of amide,  $\Delta H_{H_2O}$  is heat of hydration of amide in water and (A) is molar concentration of amide dissolved.

(2) in aqueous solution of Na-PAS :



where  $\Delta H'_{H_2O}$  is heat of hydration of amide in aqueous solution of Na-PAS,  $\Delta H''_{H_2O}$  is heat of hydration of amide-Na-PAS complex in aqueous solution of Na-PAS, and  $\Delta H'''_{H_2O}$  is heat of hydration of Na-PAS in aqueous solution of Na-PAS, and (Ap) is molar concentration of

$$\Delta H'_{\text{soln.}} - \Delta H_{\text{soln.}} = [A](\Delta H'_{H_2O} - \Delta H_{H_2O}) + (Ap)(\Delta H''_{H_2O} - \Delta H'_{H_2O} - \Delta H'''_{H_2O}) + H_c(Ap) \quad (3)$$

complex. Then in the preceding paper,<sup>1)</sup> equation (4) and (5) were assumed true, then  $\Delta H'_{\text{soln.}} - \Delta H_{\text{soln.}}$  were considered equal to  $\Delta H_c(Ap)$ .

$$\Delta H_{H_2O} = \Delta H'_{H_2O} \quad (4)$$

$$\Delta H_{H_2O} = \Delta H'_{H_2O} + \Delta H'''_{H_2O} \quad (5)$$

When amides are dissolved into a strong electrolyte aqueous solution such as sodium chloride, sodium sulfate, sodium acetate, and sodium citrate, the heat of solution of amides are coincident to the heat of solution in water as shown in Table III. So equation (4) are judged true for the case of dissolving amide in aqueous solution of Na-PAS.

TABLE III. Heats of Solution of NA and PyA in Water and in a Strong Electrolyte 20% Aqueous Solution (Kcal./mole.)

Solute \ Solvent	NA	PyA	Solute \ Solvent	NA	PyA
H <sub>2</sub> O	4.78	5.95	Na citrate 0.67M	4.65	5.97
NaCl 3.39M	4.33	5.25	Na <sub>2</sub> SO <sub>4</sub> 1.40M	4.61	5.80
CH <sub>3</sub> COONa 2.44M	4.83	5.98			

There are no data to judge equation (5), so another experiment will be made to make sure this relation.

### Experimental

**Material**—Amides and Na-PAS : Same as described in the previous paper.<sup>1)</sup> NaCl, Na<sub>2</sub>SO<sub>4</sub>, CH<sub>3</sub>COONa, Na-citrate; JIS. guaranteed reagent.

**Apparatus and Procedure**—Spectrophotometry : Same as described in the previous paper.<sup>4)</sup> Colorimetry : Same as described in the previous paper.<sup>1)</sup>

### Summary

Dissociation constants of the complex between pyrazine- or pyridine-monocarboxamide and Na-PAS in aqueous solution (*K*) were obtained at various temperatures (*T*) by the absorption spectra measurement, then heats of complex formation  $\Delta H_c$  were evaluated from the correlation between  $\log K$  and  $1/T$ . Thus obtained  $\Delta H_c$  value by the spectral method exceedingly differs from that<sup>1)</sup> thermal method considering the experimental error, so the discussion was made on this reason.

(Received May 23, 1961)

4) Part 1 : H. Negoro *et al.* : This Bulletin, 7, 91 (1959).

UDC 577.152:576.851.4:547.466.46

**131. Yukio Kameda, Katsuhiko Matsui, Yukio Kimura, Etsuko Toyoura, and Eiji Takeuchi : Studies on Acylase Activity and Microorganisms. XVII.\*<sup>1</sup>**  
Optical Resolution of Tryptophan, 2-Aminohexanedioic Acid, and 2-Aminoöctanoic Acid by Metabolism of Soil Bacteria on Benzoyl Derivatives of DL-Amino Acids.

(Laboratory of Antibiotics, Faculty of Pharmacy, Kanazawa University\*<sup>2</sup>)

In previous papers<sup>1-5)</sup> it was reported that lysine, methionine, phenylalanine, leucine, threonine, glutamic and aspartic acids, 3-(*p*-methoxyphenyl)alanine, and 3-(3,4-methylenedioxyphenyl)alanine could be assymmetrically resolved by the metabolism of soil bacteria such as KT-230 and KT-231 on benzoyl derivatives of DL-amino acids. The present work is a direct continuation and extension of the same work previously reported.

Tryptophan is one of essential amino acids, but 2-aminohexanedioic acid and 2-aminoöctanoic acid have not been isolated from protein hydrolysates. D-2-Aminohexanedioic acid was obtained as a hydrolysis product of an antibiotic, cephalosporin N.<sup>6)</sup>

\*<sup>1</sup> This work was presented at the Kantō Local Meeting of the Pharmaceutical Society of Japan, October 3, 1959. Part XVI : Ann. Rept. Fac. Pharm. Kanazawa Univ., 9, 20 (1959).

\*<sup>2</sup> Tsuchitoribanaga-machi, Kanazawa (亀田幸雄, 松井勝彦, 木村行男, 豊浦悦子, 竹内栄治).

1) Y. Kameda, E. Toyoura, K. Matsui, Y. Kimura, S. Kitagawa : Nature 182, 453 (1958).

2) Y. Kameda, E. Toyoura, K. Matsui : This Bulletin, 7, 702 (1959).

3) E. Toyoura : *Ibid.*, 7, 785 (1959).

4) *Idem* : *Ibid.*, 7, 787 (1959).

5) *Idem* : *Ibid.*, 7, 789 (1959).

6) G.G.F. Newton, E.P. Abraham : Biochem. J., 58, 103 (1954).